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#### (57) Abstract

Polypeptides capable of forming antigen binding structures specific for Rhesus D antigens include the sequences indicated in the figures 1a to 16b. The obtained polypeptides, being Fab fragments, may be used directly as an active ingredient in pharmaceutical and diagnostic compositions. The Fab and their DNA sequences can also be used for the preparation of complete recombinant Anti-Rhesus D antibodies. Useful in pharmaceutical and diagnostic compositions.

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# Polypeptides capabl of forming antigen binding structures with specificity for the Rhesus D antigens, th DNA ncoding th m and the process for their preparation and use

This invention relates to polypeptides forming antigen binding

structures with specificity for Rhesus D antigens and especially to Fab
molecules with specificity for the Rhesus D antigen. The invention also
relates to their application to provide pharmacological and diagnostic
compositions. The above Fab fragments when genetically engineered to be
part of complete antibodies are useful for the prophylaxis of hemolytic
disease of the newborn (HDN). This invention provides the novel DNA and
amino acid sequences of the above polypeptides.

Thus, the antibodies can be used for the protection of Rhesus negative women before or immediately after the birth of a Rhesus positive child to prevent HDN in subsequent pregnancies.

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The invention also includes the application of the said Fab molecules either alone or in combination with Fc constant regions as complete antibodies for the purposes of treating other illnesses which might benefit from anti-Rhesus D immunoglobulin e.g. treatment of idiopathic thrombocytopenic purpura (ITP).

In addition anti-Rhesus D immunoglobulin can be used after mistransfusions of Rhesus positive blood to Rhesus negative recipients in order to prevent sensitization to the Rhesus D antigen. Further the invention relates to the application of these Fab fragments and antibodies as diagnostic reagents.

HDN is the general designation for hemolytic anemia of fetuses and newborn babies caused by antibodies of the mother. These antibodies are directed against antigens on the surface of the fetal erythrocytes. These antigens can belong to the Rhesus, ABO or other blood group systems.

The Rhesus blood group system includes 5 major antigens: D, C, c, E and e (Issitt, P.D., Med. Lab. Sci. 45:395, 1988). The D antigen is the most important of these antigens as it is highly immunogenic eliciting anti-Rhesus D antibodies during Rhesus incompatible pregnancies and following transfusion of Rhesus incompatible blood. The D antigen is found in approximately 85% of Caucasians in Europe and those individuals are said to

be Rhesus positive. Individuals lacking the D antigen are called Rhesus negative. The expression of the D antigen can vary due to either low antigen density, hereafter known as weak D or D<sup>u</sup>, or due to partial antigenicity, hereafter known as partial D antigens.

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The Rhesus D antigen, a membrane protein of the erythrocyte, has recently been cloned and its primary structure described (Le Van Kim, C., et al., PNAS 89:10925, 1992). Modeling studies suggest that the Rhesus D antigen has 12 transmembrane domains with only very short connecting regions extending outside the cell membrane or protruding into the cytoplasm.

The partial D phenotypes were first identified in people who carried D antigen on their red cells but who had an alloanti-D in their sera (Rose, R. R. and Sanger, R., Blood groups in man, Blackwell Scientific, Oxford, U.K. 1975; Tippett, P. et al., Vox Sanguinis. 70:123, 1996). This can be explained by regarding the D antigen as a mosaic structure with at least 9 different epitopes (epD1 to epD9). Thus in some D variant people the red cells lack part of this mosaic and antibodies are made to the missing D epitopes. Rhesus positive individuals that make antibodies against partial D antigens have been classified into six main different categories (DII to DVII) each having a different abnormality in the D antigen. More recently it has been shown that these D categories gave different patterns of reaction when tested against panels of human monoclonal anti-D antibodies (Tippett, P., et al., Vox Sanguinis. 70:123, 1996). The different reaction patterns identified the 9 epitopes and so define the different partial D categories. The number of epitopes present on the D antigen varies from one partial D category to another with the  $D^{VI}$  category expressing the least, epD3, 4 and 9. The  $D^{VI}$ category is clinically important as a DVI woman can be immunized strongly enough to cause hemolytic disease of the newborn.

The prophylactic efficacy of anti-RhD IgG for prevention of hemolytic disease of the newborn is well established and has been in routine use for many years. As a result this severe disease has become a rarity. Nevertheless the underlying cause of the disease, i.e. RhD incompatibility between a RhD negative mother carrying a RhD positive child still remains and thus requires a continual supply of therapeutic anti-RhD IgG.

In recent years the assurance of a continual supply of anti-RhD lgG has become an increasing problem. The pool of available hyperimmune

serum from alloimmunized multiparous Rhesus negative women has drastically decreased due to the success of prophylactic anti-RhD. Thus the current methods of production require repeated immunization of an increasingly reluctant pool of donors for the production of high titer antiserum (Selinger, M., Br. J. Obstet. Gynaecol. 98:509, 1991). There are also associated risk factors and technical problems such as the use of Rhesus positive red blood cells for repeated immunization carrying the risk of transmission of viral diseases like hepatitis B, AIDS and other as yet unknown viruses (Hughes-Jones, N.C., Br. J. Haematol. 70:263, 1988). Therefore an alternative method for production of anti-RhD antibodies is required.

In the past few years various alternative sources of hyperimmune serum have been tried but all are associated with disadvantages. Epstein Barr Virus (EBV) transformation of lymphocytes creating B lymphoblastoid cell lines that secrete specific antibody including against the Rhesus D antigen (Crawford et al., Lancet. 386:Feb.19th, 1983) are unstable and require extensive cloning. Also due to the low transformation efficiencies (1-3% of B cells) only a restricted range of antibody specificities can be obtained from the potential repertoire. Additionally it seems that mice do not respond to the Rhesus D antigen and thus no murine monoclonal antibodies are available which could be used for producing chimaeric or humanised antibodies. Until recently the only other alternative was production of human antibodies by the hybridoma technique which was also restricted by the lack of a suitable human myeloma cell fusion partner (Kozbor, D. and Roder, J.C., Immunol. Today, 4:72, 1983).

It is thus the object of the present invention to provide Fab fragments having a reactivity against the Rhesus D antigen as well as complete antibodies comprising the Fab fragments which are free from the above mentioned drawbacks.

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In the last few years the technique of repertoire cloning and the construction of phage display libraries has opened up new possibilities to produce human antibodies of defined specificity (Williamson, R.A. et al., PNAS 90:4141, 1993). These methods were thus applied to the preparation of polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens, especially of Fab fragments having an activity against Rhesus D and partial D antigens.

The generation of human antibodies by repertoire cloning as described in recent years (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991) is based on isolating mRNA from peripheral B cells. This method offers the tools to isolate natural antibodies. 5 autoantibodies or antibodies generated during the course of an immune response (Zebedee, S.L., et al., PNAS 89:3175, 1992; Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). This method relies on constructing a recombinant antibody library from a particular donor starting from the mRNA coding for immunoglobulin (Ig) molecules. As only the peripheral blood lymphocytes (PBL) can be isolated from a donor the chances of finding specific antibody producing B cells in the periphery are increased if an individual is boosted with the desired antigen shortly before harvesting the PBL (Persson, M.A.A., et al., PNAS 88:2432, 1991). The total RNA is then isolated and the mRNA of the Ig repertoire can be cloned using Ig specific primers in the polymerase chain reaction (PCR) followed by the co-expression of heavy and light chains of the Ig molecule on the surface of a filamentous phage particle thereby forming an "organism" that in analogy to a B cell can bind to an antigen. In the literature this method is also known as the combinatorial approach as it allows the independent combining of heavy and light chains to form a functional Fab antibody fragment attached to one of the tail proteins, called plll, of a filamentous phage. Phages carrying the Fab molecules (hereafter known as Phab particles) are selected for the desired antigen specificity, by a process known as bio-panning. The antigen can be applied to a solid support, specific Phab bind to the antigen whilst non specific Phab are washed away and finally the specific Phab are eluted from the solid support. The specific Phab are then amplified in bacteria, allowed to re-bind to the antigen on the solid support and the whole process of bio-panning is repeated.

The successive rounds of panning and amplification of selected Phab in bacteria result in an enrichment of specific Phab that can be seen from a rise in titer of colony forming units (cfu) plated out after each round of panning. Our previous experience and published data indicate that specific phage can usually be detected after 4 to 6 panning rounds (Vogel, M. et al., Eur. J. Immunol. 24:1200, 1994). In the above cited related art there is however, no hint that the indicated steps can be used for a successful preparation of Fab fragments of anti-Rh D antibodies.

In the appended figures 1a to 16b; DNA sequences coding for variable regions (V regions) of anti Rh D Fab fragments and the corresponding polypeptide sequences are disclosed.

Fig. 17 shows the pComb3 expression system used according to the present invention.

Figs. 18 and 19 show the separate preparation of genes of the heavy and light chains of the complete antibody according to the description in example 6.

Subjects of the present invention are polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens according to the definition of claim 1. The table in claim 1 refers to the appended figures. The identification number for each sequence is given. The locations of the Rhesus D specific CDR1 (complementarity determining region 1), CDR2 and CDR3 regions are indicated in the figures and according to base pair number in the table of claim 1. Preferred polypeptides according to the invention are anti-Rhesus D antibodies which include the variable regions of the heavy and light chains according to the sequences given in Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the variable regions of the light chain.

Further subjects of the present invention are the DNA sequences coding for antigen binding polypeptides according to the definition of claim 6. Prefered DNA sequences are those coding for variable regions of Fab fragments of anti-Rh D antibodies according to the Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the heavy chain and the Figs. 1b, 2b, ... 16b are related to the light chain.

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A further subject of the present invention is a process for preparing recombinant Fab polypeptides according to the definition in claim 11.

A further subject of the present invention is a process for the selection of recombinant polypeptides according to claim 12.

Further subjects of the present invention are anti-Rh D antibodies according to the definition of claim 14, preferably anti-Rh D immunoglobulin molecules comprising the heavy and light chain variable regions according to

the Figs. 1a to 16b combined with known heavy and light chain constant regions.

Further subjects of the present invention are pharmaceutical and diagnostic compositions comprising polypeptides, anti-Rh D antibodies or Fab fragments according to the invention.

The total re-amplified Phab population obtained after each panning can be tested for specificity using various methods such as ELISA and immunodot assays. It is also defined by the nature of the antigen e.g. anti-Rhesus D Phabs are detected by indirect haemagglutination using a rabbit anti-phage antibody or equivalent Coombs reagent as the cross linking antibody. Once a total Phab population has been identified as positive for the desired antigen, individual Phab clones are isolated and the DNA coding for the desired Fab molecules is sequenced. Individual Fab can then be produced by use of the pComb3 expression system which is illustrated in Fig. 16. In this system the gIII gene, coding for the tail protein pIII, is cut out from the phagemid vector pComb3. This allows production of soluble Fab in the bacterial periplasm. Such individual Fab fragments can then be tested for antigen specificity.

The phage display approach has also been used as a means of rescuing monoclonal antibodies from unstable hybridoma cell lines. This has been reported for anti-Rhesus D antibodies (Siegel, D.L. and Silberstein, L.E., Blood. 83:2334, 1994; Dziegiel, M. et al., J. Immunol. Methods. 182:7, 1995). A phage display library constructed from non-immunized donors has also been used to select Fv fragments (i.e. variable regions of heavy and light chains, V<sub>H</sub> and V<sub>L</sub>) specific for human blood group antigens which included one Fv fragment reacting against the Rhesus D antigen (Marks, J.D. et al., Biotechnology. 11:1145, 1993).

Important considerations when constructing combinatorial libraries are the source of cells used for RNA extraction and the nature of the antigen used for panning. Therefore, this invention uses a hyperimmune donor who was boosted i.v. with Rhesus D\* red blood cells (rbc). The PBL of the donor were harvested at +5 and +18 days after the i.v. boost and were used to construct 2 combinatorial libraries hereafter known as library D1 (LD1) and library D2 (LD2) respectively. Double immunofluorescence analysis of the harvested PBL, using the markers CD20 and CD38 for pan B cells and

lymphoblastoid cells respectively, showed a higher than normal percentage of lymphoblastoid B cells, of plasma cell morphology. The high number of plasma cells found in the peripheral blood is most unusual as normally there are less than 1% in the periphery and probably indicates that the donor had a high percentage of circulating B cells with specificity for the Rhesus D antigen.

After construction of the library, the selection of Phabs specific for the Rhesus D antigen was achieved by bio-panning on fresh whole rbc of phenotype R1R1 (CDe/CDe) i.e. the reference cells used for Rhesus D typing. This was necessary since the Rhesus D antigen, an integral membrane protein of 417 amino acids (Le Van Kim, C. et al, PNAS 89:10925, 1992), loses its immunogenicity during purification (Paradis, G. et al, J. Immunol. 137:240, 1986) and therefore a chemically purified D antigen cannot be bound to a solid phase for selection of immunoreactive Phabs as for other antigen specificities previously selected in this system (Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). Modelling studies have suggested that only very short connecting regions of the Rhesus D antigen extend outside the cell membrane or protrude into the cytoplasm (Chérif-Zahar, B. et al, PNAS 87:6243, 1990). Thus the parts of the RhD antigen visible to antibodies are relatively restricted and may be under conformational constraint. This aspect of the Rhesus D antigen becomes even more important when considering selection of Phabs with reactivity against the partial D phenotypes which essentially lack certain defined epitopes of the D membrane protein (Mouro, I. et al, Blood. 83:1129, 1994).

Furthermore, since whole rbc do not only express the D antigen, a series of negative absorptions had to be performed on Rhesus D negative rbc in order to absorb out those Phabs reacting with the other antigenic proteins found on the rbc.

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This panning procedure performed on Phabs coming from both LD1 and LD2 librairies resulted in the isolation of 6 different Fab producing clones from library LD1, 8 different Fab producing clones from library LD2 and 2 Fab producing clones from the pooled libraries LD1 and LD2.

The nomenclature and the figures where the sequences are listed are given in table 1.

Table 1

LIBRARY LD1 Clone No.	V <sub>H</sub> - Sequence Figure	V <sub>L</sub> - Sequence Figure	LIBRARY LD2 Clone No.	V <sub>H</sub> - Sequence Figure	V <sub>L</sub> - Sequence Figure
LD1-40	1a	1b	LD2-1	6a	6b
LD1-52	2a	2b	LD2-4	7a	7b
LD1-84	3a	3b	LD2-5	8a	8b
LD1-110	4a	4b	LD2-10	9a	9b
LD1-117	5a	5b	LD2-11	10a	10b
			LD2-14	11a	11b
			LD2-17	12a	12b
			LD2-20	13a	13b

The above Fab clones show exclusive reactivity against the Rhesus D antigen, 3 of 5 D<sup>u</sup> rbc tested and agglutinating reactivity against the Partial D phenotypes as follows: Rh33, DIII, DIVa, DIVb, DVa, DVII,

However, using the above mentioned R1R1 rbc for panning of the Phabs, no clones were isolated which reacted against the Partial DVI phenotype. As the serum of the original hyperimmune donor tested at the time of construction of the recombinant library, was known to react against the DVI phenotype the recombinant library should also contain the anti-DVI specificity.

In order to select for the DVI reactivity the panning conditions were changed in that different cells were used. A special donor whose rbc had been typed and were known to express the Partial DVI phenotype was used as the source of cells for re-panning the LD1 and LD2 libraries. This second series of pannings was essentially performed in the same way as the first series except for the substitution of DVI rbc for R1R1 rbc and the addition of bromelase treatment to the DVI rbc. The DVI phenotype expresses the least number of Rhesus D epitopes and it is therefore difficult to make antibodies against it. It has been reported that only 15% of unselected polyclonal anti-D and 35% of selected anti-D made by Rhesus D negative subjects reacted with DVI+ cells (Mouro, I. et al, Blood. 83:1129, 1994). Bromelase treatment which removes N- acetylneuraminic acid (sialic acid) from the rbc membrane, was performed in order to render the Rhesus DVI epitopes more accessible during the panning with the pre-absorbed Phabs.

This second series of pannings on the LD1 library resulted in 1 Fab producing clone LD1-6-17. The nomenclature is given in table 2.

Table 2

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LIBRARY LD1	V <sub>H</sub> -Sequence figure	V <sub>L</sub> -Sequence figure
Clone No: LD1-6-17	14a	14b

However this clone was reacting with Rhesus alleles C and E and showing a false positive reaction with DVI positive rbc. This was also due to the phenotype of the DVI donor (Cc DVI ee) who expressed the C allele which was not absorbed out by the Rhesus negative rbc (ccddee).

Thus a third series of pannings on a pool of the LD1 and LD2 libraries was performed using different rbc for the absorption phase. After 6 rounds of panning using both bromelase treated and non treated rbc for both the absorption steps and the elution from DVI positive rbc a total population of Phabs was obtained which reacted exclusively with rbc of phenotype R1R1 (CCDDee) and 2 different donors expressing the DVI variant.

This third series of pannings on the LD1 and LD2 librairies resulted in 2 Fab producing clones reacting with DVI+ rbc. The nomenclature is given in table 3.

Table 3

LIBRARY LD1/LD2	V <sub>H</sub> -Sequence figure	V <sub>L</sub> -Sequence figure
Clone No: LD1/2-6-3	15a	15b
Clone No: LD1/2-6-33	. 16a	16b

Thus a total of 16 different anti-Rhesus D Fab clones have been isolated. The DNA from these clones has been isolated and sequenced using Fluorescent Cycle Sequencing on an ABI 373A Sequencing System. The nucleotide and corresponding amino acid sequences of the said Fab clones form the basis of this invention.

Sequence analysis has revealed that several clones were isolated bearing the same  $V_H$  gene segment but different  $V_L$  gene segments. This is

the case for the two clones LD2-1 and LD2-10, for the two clones LD2-4 and LD2-11, and for the three clones LD2-14, LD1/2-6-3 and LD1/2-6-33, respectively.

The DNA sequences obtained and Fab fragments are useful for the preparation of complete antibodies having an activity against the Rhesus D antigen. Suitable expression systems for such antibodies are mouse myeloma cells or chinese hamster ovary cells.

The examples which follow explain the invention in detail, without any restriction of the scope of the invention.

Example 1 describes the construction of 2 combinatorial librairies; especially the aforementioned LD1 and LD2 libraries.

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Example 2 describes a series of pannings using R1R1 rbc on the said LD1 and LD2 libraries in detail.

Example 3 describes a series of pannings using both bromelase and non bromelase treated rbc for absorption and bromelase treated DVI positive rbc using a pool of the said LD1 and LD2 librairies.

Example 4 describes an indirect haemagglutination assay using a rabbit anti-phage antibody, as an equivalent Coombs reagent, to monitor the enrichment and specificity of Rhesus D specific Phabs after panning.

Example 5 describes the preparation and purification of Fab antibody fragments for application as diagnostic reagents.

Example 6 describes the preparation of complete anti-Rhesus D immunoglobulins using the sequences of the present invention.

#### Example 1

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WO 97/49809

# Construction of the recombinant LD1 and LD2 libraries

### a) Source of the lymphocytes

A male adult who was a member of the volunteer pool of
hyperimmune Rhesus D donors was given an i.v. boost of 2 ml of packed rbc
from a known male donor of blood group O RhD\*. The PBL were harvested at
+5 and +18 days after the boost and the mononuclear cells (MNC) isolated by
Ficoll gradient centrifugation (Lymphoprep, Pharmacia, Milwaukee, WI). The
results of donor lymphocyte analysis of day +5 are given in table 4. The +5
day MNC were used directly for RNA preparation using a phenol-chloroform
guanidinium isothiocyanate procedure (Chomczynski, P. and Sacchi, N.,
Anal. Biochem. 162:156, 1987). The +18 day MNC were first cultured for 3
days in RPMI-1640 medium (Seromed, Basel) containing 10 U/ml of IL-2
(Sandoz Research Center, Vienna, Austria) and 10 μg/ml of pokeweed
mitogen (PWM; Sigma L9379, Buchs, Switzerland) before extracting RNA.

Table 4
Immunofluorescence analysis of donor lymphocytes +5 days
after rbc i.v. boost

Cell surface antigen	% Positive cells	Cell surface antigen	% Positive cells	
CD20	15	CD8	12	
CD38	20	CD25	7.6	
CD20/38	. 15	CD57	12.5	
· ·	47	CD14	6	
CD3	34	HLA-DR	18	
CD4				

## b) Construction of Library

Two separate libraries were constructed called LD1 and LD2 (as detailed above) corresponding to the cells harvested at +5 days and +18 days (finally +21 days including the +3 days PWM stimulation) after the i.v. boost respectively. Total RNA was then prepared from these cells using a phenol-chloroform guanidinium isothiocyanate method. From this RNA, 10  $\mu$ g were

used to make cDNA using an oligo(dT) primer (400 ng) and reverse transcribed with M-MuLV reverse transcriptase according to the conditions specified by the supplier (Boehringer Mannheim Germany). PCR amplification was performed as described in Vogel, M. et al., E.J. of Immunol. 24:1200, 1994. Briefly, 100 µl PCR reaction contained Perkin-Elmer buffer with 10 mM MgCl<sub>2</sub>, 5 μl cDNA, 150 ng of each appropriate 5' and 3' primer, all four dNTP at 200  $\mu\text{M}$  each and 2 U/ml Taq Polymerase (Perkin Elmer, NJ). The PCR amplification of the heavy and light chains of the Fab molecule was performed separately with a set of primers from Stratacyte (details given below). For the 10 heavy chain six upstream primers were used that hybridize to each of the six families of the V<sub>H</sub> genes whereas one kappa and one lambda chain primer were used for the light chain. The downstream primers were designed to match the hinge region of the constant domains  $\gamma 1$  and  $\gamma 3$  for the heavy chain. For the light chain the downstream primers were matched to the  $3^\prime$  end of kappa and lambda constant domains. The heavy and light chain PCR products were pooled separately, gel purified and cut with Xho1/Spe1 and Sac1/ Xba1 restriction enzymes (Boehringer Mannheim), respectively. After digestion the PCR products were extracted once with phenol: chloroform: isoamylalcohol and purified by gel excision. The insertion of the Xho1/Spe1 digested Fd fragment and subsequent ligation of the Sac1/Xba1 digested light chain into the pComb3 vector, the transformation into XL1-Blue cells, and the production of phages were performed as described by (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991).

After transformation of the XL1-Blue E.coli cells samples were withdrawn and titrated on plates to determine the library size. These results indicated expression libraries of 7.5x10<sup>6</sup> and 7.7x10<sup>6</sup> cfu (colony forming units) for LD1 and LD2 respectively.

#### c) PCR Primers

VHI 5'-CAC TCC CAG GTG CAG CTG CTC GAG TCT GG-3'
VHII 5'-GTG CTG TCC CAG GTC AAC TTA CTC GAG TCT GG-3'
VHIV 5'-GTC CAG GTG GAG GTG CAG CTG CTC GAG TCT GG-3'
VHV 5'-GTC CTG TCC CAG GTG CAG CTG CTC GAG TCT GG-3'
VHVI 5'-GTC TGT GCC GAG GTG CAG CTG CTC GAG TCT GG-3'
VHVI 5'-GTC CTG TCA CAG GTA CAG CTG CTC GAG TCA GG-3'
CHI(gl) 5'-AGC ATC ACT AGT ACA AGA TTT GGG CTC-3'

- VL(k) 5'-GT GCG AGA TGT GAG CTC GTG ATG ACC CAG TCT CAA GCT
- CL(k) 5'-T CCT TCT AGA TTA CTA ACA CTC TCC CCT GTT GAA GCT CTT TGT GAC GGG CGA ACT C-3'
- VL(I) 5'C TGC ACA GGG TCC TGG GCC GAG CTC GTG GTG ACT CA-3'
- 5 CL(I) 5'G CAT TCT AGA CTA TTA TGA ACA TTC TGT AGG GGC-3'

### d) Vectors and bacterial strains

The pComb3 vector used for cloning of the Fd and the light chain was obtained from the Scripps Research Institute La Jolla, CA; (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991). The *Escherichia coli* strain XL1-Blue used for transformation of the pComb3 vector and the VCSM13 helper phage were purchased from Stratacyte (La Jolla, CA).

#### Example 2

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# Selection of Rhesus D Phabs from LD1 and LD2 libraries on R1R1 rbc

### a) Absorption and Bio-Panning

A series of three negative absorptions on rbc group O Rh negative were performed for each panning round before positive selection on rbc group O Rh positive (R1R1). Fresh rbc were collected in ACD (acid citrate dextrose) anticoagulant and washed 3 times in 0.9% NaCl. The rbc were counted in Hayems solution and adjusted to  $40x10^6/ml$ . Absorption : 1 ml of phage preparation in PBS/3%BSA was added to rbc group O Rh negative pellet (16x10<sup>6</sup> rbc) in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated twice more. After the final absorption the phage supernatant was added to the rbc group O Rh positive pellet (16x10 rbc) and again incubated at RT for 30 min. with gentle shaking. Then the rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min. 300 x g at 4°C, followed by elution with 200  $\mu$ l of 76 mM citric acid pH 2.8 for 6 min, at R.T. and neutralisation with 200 μl 1M Tris. The rbc were centrifuged 300 x g, 5 min. at 4°C and the resulting supernatant containing the eluted phages was carefully removed and stored with carrier protein

(0.3% BSA) at 4°C ready for re-amplification. The numbers of Rhesus D specific Phabs of each panning round are given in table 5.

Table 5

Selection of Rhesus D+ Phabs from the LD1 and LD2 libraries on R1R1 rbc

	No. of eluted Rhesus D specific phages			
Panning Round No. <sup>a)</sup>	Library D1 cfu	Library D2 cfu		
1	8x10 <sup>6</sup>	4.6x10 <sup>7</sup>		
2	6x10 <sup>7</sup>	1.4x10 <sup>7</sup>		
3	1x10 <sup>8</sup>	7.9x10 <sup>7</sup>		
4	3x10 <sup>8</sup>	1.3x10 <sup>8</sup>		
5	3x10 <sup>8</sup>	1x10 <sup>8</sup>		
6	nd	2.8x10 <sup>8</sup>		

a) For each round 10<sup>12</sup> Phabs were incubated in tubes with rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus positive (R1R1)

nd = not done

cfu = colony forming units

#### Example 3

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# Selection of Rhesus D Phabs from the pooled LD1 and LD2 libraries on DVI+ rbc

a) Absorption on rbc group O Rh negative, phenotypes 1 (r'r, Ccddee) and 2 (ryry, CCddEE)

A series of four negative absorptions on rbc group O Rh negative was performed for each panning round before positive selection on rbc group O Rh DVI positive. The negative absorptions were performed in the following order: Step 1) phenotype 1 treated with bromelase; step 2) phenotype 1 no bromelase; step 3) phenotype 2 treated with bromelase; step 4) phenotype 2

no bromelase. Frozen rbc were thawed into a mixture of sorbit and phosphate buffered saline, left standing in this solution for a minimum of 10 min. and then washed 5 to 6 times in phosphate buffered saline and finally stored in stabilising solution (DiaMed EC-Solution) ready for use. Before panning the rbc were washed 3 times in 0.9% NaCl. followed by counting in Hayems solution. Absorption: 1 ml of phage preparation in PBS/3%BSA was added to an rbc pellet (2x10<sup>8</sup>) as in step 1 in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated using rbc as detailed above in steps 2, 3, and 4.

# b) Treatment of rbc Rhesus D negative r'r and ryry and Rhesus DVI+ with bromelase

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Bromelase 30 (Baxter, Düdingen, Switzerland) was used to treat rbc Rhesus DVI+ in the same proportions as used in a routine haemagglutination assay, i.e. 10 μl bromelase per 2x10<sup>6</sup> rbc. Thus bromelase was added to the required amount of rbc and incubated at 37°C for 30 min. followed by washing 3 times in 0.9% NaCl, re-counting in Hayems solution and adjusting to the required concentration in PBS/3% BSA ready for Phab panning.

## c) Bio-Panning on bromelase treated Rhesus DVI+ rbc

After the final absorption on rbc ryry non bromelase treated the phage supernatant was divided into 2 equal parts and added either to the enzyme or non enzyme treated rbc group O Rh DVI+ pellet  $(40 \times 10^6)$  respectively and again incubated at RT for 30 min. with gentle shaking. Then the 2 populations of rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min.  $300 \times g$  at 4°C, followed by elution with  $200 \mu l$  of 76 mM citric acid pH 2.8 for 6 min. at R.T. and neutralisation with  $200 \mu l$  1M Tris. The rbc were centrifuged  $300 \times g$ , 5 min. at 4°C and the resulting supernatants containing the eluted phages from either the bromelase or non bromelase treated DVI+rbc were carefully removed and stored with carrier protein (0.3% BSA) at 4°C ready for re-amplification. In further rounds of panning the eluted phage from either the bromelase or non bromelase treated DVI+rbc were

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kept separate and each followed the absorption protocol steps 1 to 4. The elution step was slightly different compared to panning round 1 as the phage populations were not again divided into 2 parts. Only those phage eluted from bromelase treated DVI+ rbc were also eluted again from bromelase treated DVI+ rbc and only those phage eluted from the non bromelase treated DVI+ rbc were also again eluted from non bromelase treated DVI+ rbc. The numbers of specific Phabs after each panning round are given in table 6.

Table 6 Selection of Rhesus D Phabs from pooled LD1 and LD2 libraries on Rhesus DVI+ red blood cells

	No. of eluted Rhesus DVI+ specific phages				
Panning Round No.a)	- Bromelase cfu	+ Bromelase cfu			
1	1.9x10 <sup>6</sup>	4.4x10 <sup>6</sup>			
2	1.6x10 <sup>6</sup>	4x10 <sup>5</sup>			
3	2.4x10 <sup>7</sup>	4.1x10 <sup>7</sup>			
4	3x10 <sup>6</sup>	5x10 <sup>7</sup>			
5	1x107 <sup>8</sup>	1x10 <sup>8</sup>			
6	: nd	3x10 <sup>8</sup>			

a) For each round 10<sup>12</sup> Phabs were incubated in tubes with 2 different phenotypes of rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus DVI+.

#### Example 4

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Monitoring of the panning rounds and determination of the specificity of the enriched Phabs using a rabbit anti-phage antibody

Indirect haemagglutination assay

Freshly collected rbc of different ABO and Rhesus blood groups were washed 3 times in 0.9% NaCl and adjusted to a 3-5% solution (45-  $50x10^7$ /ml) in either 0.9% NaCl or PBS/3% BSA. For each test condition  $50~\mu$ l rbc and 100  $\mu$ l test (precipitated and amplified phage or control antibodies) were incubated together in glass blood grouping tubes (Baxter, Düdingen, Switzerland) for 30 min. at 37°C. The rbc were washed 3 times in 0.9% NaCl

and then incubated with 2 drops of Coombs reagent (Baxter, Düdingen, Switzerland) for positive controls or with 100 µl of 1/1000 diluted rabbit antiphage antibodies (made by immunising rabbits with phage VCSM13 preparation, followed by purification on an Affi-Gel Blue column and absorption on E. coli to remove E. coli-specific antibodies). The tubes were incubated for 20 min at 37°C, centrifuged 1 min at 125xg and rbc examined for agglutination by careful shaking and using a magnifier viewer.

When purified Fab were tested for agglutination, an affinity purified anti-Fab antibody (The Binding Site, Birmingham, U.K.) was used instead of the rabbit anti-phage antibody.

Table 7 shows the results of haemagglutination tests of Phab samples after different panning rounds on R1R1 rbc.

Table 8 shows the results of haemagglutination tests of Phab samples after different panning rounds on Rhesus DVI+ rbc.

Table 9 shows the reactivity pattern of individual Fab clones from libraries LD1 and LD2 with partial D variants.

Table 7 Monitoring of Phabs from LD1 and LD2 libraries by indirect haemagglutination after panning on R1R1 rbc

haemagglutination	on after pariting of		
Phab sample	Library LD1	Library LD2	
Panning round	tested on rbc O Rh D+ (a)		
No. 4			
undiluted	+	+	
1/4	+	+/-	
1/20	-	-	
No.5			
undiluted	++	+	
1/4	++	+	
1/20	-	-	
No. 6		+++	
undiluted	nd		
1/4	nd	++	
1/20	nd	nd	
Helper phage (b)			
undiluted, 1/4, 1/20	-		

a ) Indirect haemagglutination was performed in glass tubes using 50  $\mu$ l rbc (40x10 $^7$ /ml) and 100  $\mu$ l Phabs starting at 4x10 $^{11}$ /ml. After 30 min. at 37°C the

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rbc were washed 3 times and further incubated for 20 min. at 37°C with a 1/1000 dilution of rabbit anti-phage antibody.

- b) The M13 helper phage was used as a negative control and showed no non-specific agglutination due to the phage particle alone.
- Agglutination was scored by visual assessment from +++ (strong agglutination) descending to (no agglutination). nd = not done

Table 8 Monitoring of Phabs from pooled LD1 and LD2 libraries by indirect haemagglutination after panning on Rhesus DVI+ rbc

Phab sample Panning round	rbc phenotypes					
	CCDDee	ccddee	Ccddee	CCddEE	DVI (E.J.)	DVI (K.S.)
Non Bromelase treated rbc DVI+						<i>11</i>
Round No.3	a) +++	-	+/-	(+)	+/-	+/-
Round No. 5	++	- :	-	-	<u>-</u>	-
Bromelase			· -· ·			
treated rbc DVI+		14				
Round No.4	+++	-	+/-	-	. (+)	+/-
Round No.5	+++	-	+/-	+/-	(+++)	++
Round No.6	++++			· 	+++	+++
LD1 - 6 - 17			reactive v	with C and	E	
LD1/2 - 6 - 3	++++	-	-	-	+/-	nd
LD1/2 - 6 - 33	++++			-	+	nd

a) Agglutination was scored by visual assessment from ++++ (strong agglutination) descending to - (no agglutination). nd = not done

Note: Only those Phabs eluted from bromelase treated DVI+ rbc showed evidence of agglutination against 2 different DVI+ donors.

Table 9

Clonal Analysis of R activity of Fab anti-Rhesus D Clones from Libraries

D1 and LD2 against Partial D Variants

			Parti	al D Var	iants		
<sup>(a)</sup> Fab Clone No	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD1 - 40		(b)+++	+	+	+/-	_	++
- 52	_	+++	-	-	+++	-	+++
- 84	_	++	-	-	-	-	+
- 110	(+)	+++	++	+	+	-	++
- 117	-	+++	<u>-</u> ·	-	-	-	++
LD2 - 1	+++	nd	+++	+++	+	-	+++
- 4	•	+++	•	+	-	-	+
- 5	-	nd	+++	+++	-	-	+++
- 10	(-)	+++	+++	+++	+	-	++
- 11	-	+++	-	-	-	-	++
- 14	+++	+++	+++	+++	+++	-	+++
- 17	_	+++	+++	+	+/-	•	+++
- 20	<b>.</b> ,	+++	+++	-	+/-	-	+++
LD1/2 - 6- 3	++	+++	+++	++	+++	+	++
LD1/2 - 6- 33	+/-	+++	+++	++	+++	+	++

<sup>5</sup> a) soluble Fab preparations were made of each clone followed by indirect haemagglutination.

b) Agglutination was scored by visual assessment from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

#### Example 5

# Preparation and purification of Fab antibody fragments for application as diagnostic reagents

After the bio-panning procedures detailed in Examples 2 and 3 a phage population which showed specific agglutination on Rhesus D+ rbc was selected and used to prepare phagemid DNA. More precisely the Phabs selected on R1R1 rbc were used after the 5th and 6th rounds of bio-panning for LD1 and LD2 libraries respectively and after the 5th bio-panning on DVI+ rbc for isolation of the LD1-6-17 clone. In order to produce soluble Fab, the sequence glll coding for the plll tail protein of the phage particle must be deleted.

Phagemid DNA was prepared using a Nucleotrap kit (Machery-Nagel) and the gIII sequence was removed by digesting the so isolated phagemid DNA with Nhe1/Spe1 as described (Burton, D.R., et al., PNAS, 1989). After transformation into XL1-Blue individual clones were selected (nomenclature given in table 1) and grown in LB (Luria Broth) containing 50 μ g/ml carbenicillin at 37°C to an OD of 0.6 at 600 nm. Cultures were induced with 2 mM isopropyl β-D-thiogalactopyranoside (IPTG) (Biofinex, Praroman, Switzerland) and grown overnight at 37°C. The whole culture was spun at 10,000xg for 30 min. at 4°C to pellet the bacteria. The bacterial pellet was treated with a lysozyme/DNase solution to liberate the Fab fragments inside the cells. As some Fab were released into the culture supernatant this was also harvested separately. These Fab preparations were then pooled and precipitated with 60% ammonium sulphate (Merck, Darmstadt, Germany) to concentrate the Fab followed by extensive dialysis in phosphate buffered saline (PBS) and ultracentrifugation at 200,000xg to pellet any insoluble complexes. The Fab preparations were then purified on a ceramic hydroxyapatite column (HTP Econo cartridge, BioRad, Glattbrugg, Switzerland) using a gradient elution of PBS (Buffer A) and PBS + 0.5M NaCl (Buffer B). The linear gradient was programmed to increase from 0-100% Buffer B in 40 min. The Fab was eluted as a single peak between 40-60% Buffer B. The positive fractions as identified by immunodot assay using an anti-Fab peroxidase conjugate (The Binding Site, Birmingham, U.K.) were pooled, concentrated using polyethylene glycol and extensively dialysed

against PBS. The positive fractions from the hydroxyapatite column for each clone were used in a classical indirect haemagglutination assay in glass tubes using either the standard Coombs reagent (Baxter Diagnostics AG Dade, anti-human serum) or an anti-Fab (The Binding Site, Birmingham, U.K.) as the cross linking reagent. These Fab of defined specificity on the Partial D variants as shown on page 18 can be used to type rbc of unknown Partial D phenotype.

#### Example 6

## Construction of complete immunoglobulin genes

The LD2-14 heavy chain V gene (V<sub>H</sub> gene) was amplified from the anti-Rhesus D-Fab-encoding plasmid LD2-14 with the polymerase chain reaction (PCR) using specific primers. The 5'-primer had the sequence: 5'-GGGTCGACGCACAGGTGAAACTGCTCGAGTCTGG-3', whereas the 3'-primer was of the sequence:

5 5'-GCCGATGTGTAAGGTGACCGTGGTCCCCTTG-3'.

The PCR reaction was performed with Deep Vent DNA Polymerase and the buffer solution (2mM Mg\*\*) from New England Biolabs at the conditions recommended by the manufacturer including 100 pmol of each primer and the four deoxynucleotides at a concentration of 250 μM each. The reaction was run for 30 cycles with the following temperature steps: 60 s at 94°C (extended by 2 min. during the first cycle), 60 s at 57°C and 60 s at 72°C (extended by 10 min. during the last cycle). Post-amplification addition of 3' A-overhangs was accomplished by a subsequent incubation for 10 min at 72°C in the presence of 1 unit Taq DNA Polymerase (Boehringer Mannheim, Germany). The PCR product was purified using the QIAquick PCR purification kit (Qiagen, Switzerland) and cloned into the vector pCRII using Invitrogen's TA cloning kit (San Diego, USA). Having digested the resulting plasmid TAVH14 with Sall and BstEll, the V<sub>H</sub> gene was isolated by preparative agarose gel electrophoresis using Qiagen's QIAquick gel extraction kit. 30

Vector # 150 (Sandoz Pharma, Basel) which contained an irrelevant but intact human genomic immunoglobulin  $V_{\rm H}$  gene was cut with

Sall and BstEII, and the vector fragment was isolated by preparative agarose gel electrophoresis using Qiagen's QIAquick gel extraction kit. Ligation of vector and PCR product was performed at 25°C for 2 hours in a total volume of 20 μl using the rapid DNA Ligation kit (Boehringer Mannheim, Germany). Following ligation, the reaction mix was diluted with 20 μl H<sub>2</sub>0 and extracted with 10 volumes of n-butanot to remove salts. The DNA was then pelleted by centrifugation, vacuum dried and resuspended in 10 μl H<sub>2</sub>0. 5 μl of this DNA solution were electroporated (0.1 cm cuvettes, 1.9 kV, 200 Ω, 25 μFD) with a GenePulser (BioRad, Gaithersburg) into 40 μl of electroporation competent E. coli XL1-blue MRF' (Stratagene, La Jolla), diluted with SOC medium, incubated at 37°C for 1 hour and plated on LB plates containing ampicillin (50

μg/ml). Plasmid-minipreps (Qiagen, Basel) of the resulting colonies were checked with restriction digests for the presence of the appropriate insert.

With this procedure, the irrelevant resident  $V_H$  gene in vector # 150 was replaced by the amplified anti-Rhesus D  $V_H$  sequence of LD2-14 and yielded plasmid cassVH14. The structure of the resulting immunoglobulin  $V_H$  gene construct was confirmed by sequencing, cut out by digestion with EcoRI and BamHI and gel purified as described above. Expression vector # 10 (Sandoz Pharma, Basel) containing the human genomic immunoglobulin  $C\gamma1$  gene segment was also digested with EcoRI and BamHI, isolated by preparative agarose gel electrophoresis, ligated with the EcoRI/BamHI- $V_H$  gene segment previously obtained from plasmid cassVH14 and electroporated into E. coli XL1-blue MRF' as outlined above. This resulted in a complete anti-Rhesus D heavy chain immunoglobulin gene in the expression vector 14IgG1 (Figure and ).

The LD2-14 light chain V gene (V<sub>L</sub> gene) was amplified from the same anti-Rhesus D-Fab plasmid LD2-14 by PCR using specific primers. The 5'-primer had the sequence:

5'-TACGCGTTGTGACATCGTGATGACCCAGTCTCCAT-3', whereas the 3'-primer was of the sequence:

#### 5'-AGTCGCTCAGTTCGTTTGATTTCAAGCTTGGTCC-3'.

PCR reaction, product purification and subsequent cloning steps were analogous to the steps described for the  $V_H$  gene, except that the appropriate light chain vectors were used. Briefly, the  $V_L$  PCR product was

cloned into pCRII vector yielding plasmid TAVL14, excised therefrom with  $\mathit{Mlul}$  and  $\mathit{HindIII}$  and isolated by gel extraction. The  $V_L$  gene was subsequently cloned into the  $\mathit{Mlul}$  and  $\mathit{HindIII}$  sites of vector # 151 (Sandoz Pharma, Basel) thus replacing the irrelevant resident  $V_L$  gene by the amplified anti-Rhesus D  $V_L$  sequence of LD2-14. Having confirmed the sequence of the resulting plasmid cassVL-14, the  $\mathit{EcoRII}$  Xbal fragment containing the  $V_L$  gene was then subcloned into the restriction sites  $\mathit{EcoRI}$  and Xbal of vector # 98 (Sandoz Pharma, Basel, Switzerland) which contains the human genomic immunoglobulin  $C_K$  gene segment. This procedure replaced the irrelevant resident  $V_L$  gene in plasmid # 98 and yielded the expression vector 14kappa which contains the complete anti-Rhesus D light chain immunoglobulin gene.

The mouse myeloma cell line SP2/0-Ag 14 (ATCC CRL 1581) was cotransfected by electroporation with the expression vectors 14lgG1 and 14kappa previously linearized at the unique EcoRI and NotI cleavage site, respectively. The electroporation was performed as follows: exponentially growing cells were washed twice and suspended in phosphate buffered sucrose (272 mM sucrose, 1 mM MgCl<sub>2</sub>, 7 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4) at a density of 2 x 10<sup>7</sup> cells/ml. 0.8 ml of cells were added to a 0.4 cm cuvette, mixed with 15 μg of linearized plasmids 14lgG1 and 14kappa, held on ice for 15 min., electroporated with 290 Volts, 200  $\Omega$ , 25  $\mu FD$ , put back on ice for 15 min., transferred to a T75 cell culture flask with 20 ml of cold RPMI 1640 medium (10% heat inactivated fetal bovine serum, 50 μM beta-mercaptoethanol), left for 2 h at room temperature and then incubated for 60 h at 37°C. After this period, the cells were transferred to 50 ml of medium containing 1 mg/ml G418 for selection. Stable transfectants were then selected in the presence of increasing concentrations of methotrexate to amplify the integrated DNA and thus increasing the expression of the corresponding antibody rD2-14.

Expression of rD2-14 in the culture's supernatant (SrD2-14) was monitored by an enzyme linked immuno-sorbent assay (ELISA) specific for human  $\gamma 1$  and kappa chains. Quantification of the Rhesus D specific immunoglobulins in the anti-D assay according to Ph. Eur. revealed between 1.1 and 11.4  $\mu$ g/ml of agglutinating antibody in such supernatants. They tested agglutination negative for Rhesus negative rbc and revealed the same agglutination potential against partial D variants as the Fab LD2-14 expressed in E. coli. The data are shown in table 10.

Table 10

Comparative analysis of reactivity of Fab anti-Rhesus D clone LD2-14

and antibody rD2-14 against partial D variants

				Partial D Variants					
	R1R1	rr	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD2-14	+++	_	+++	+++	+++	+++	+++	-	+++
SrD2-14	+++	-	+++	+++	+++	+++	+++	-	+++
тсв	-	-							

Agglutination was scored by visual assessement from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

LD2-14: Fab fragment prepared as described in Example 5;

SrD2-14: cell culture supernatant containing antibody rD2-14;

TCB: cell culture supernatant of untransfected cells.

#### **Claims**

1. Polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences  $V_{\rm H}$  and  $V_{\rm L}$  with the same or different identification numbers according to the figures given in the table below:

	V <sub>H</sub>				V <sub>L</sub>			
Identi- fication	Figure	CDR 1 base pair No.	CDR 2	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.
No. LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288
	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285
LD1-84		91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285
LD1-110	Fig. 4a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288
LD1-117	Fig. 5a		148-198	295-342	Fig. 6b	61-99	145-165	262-294
LD2-I	Fig. 6a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282
LD2-4	Fig. 7a	91-105		295-342	Fig. 8b	64-96	142-162	259-288
LD2-5	Fig. 8a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294
LD2-10	Fig. 9a	91-105	148-198	296-343	Fig. 10b	<del>                                     </del>	142-162	259-285
LD2-11	Fig. 10a	91-105	148-198				142-162	259-285
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	<del></del>	142-162	259-285
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	<del> </del>	142-162	259-285
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b			259-285
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b		142-162	
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b		142-162	259-285
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285

- 2. Polypeptides according to claim 1 which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences  $V_{\rm H}$  and  $V_{\rm L}$  with the same identification numbers according to the figures given in the table of claim 1.
- 3. Polypeptides according to claim 1 which include regions with the amino acid sequences  $V_H$  and  $V_L$  and have identification numbers according to the figures given in the table of claim 1.

- 4. Polypeptides according to claim 1, 2 or 3 characterised as antigen binding Fab fragments.
- 5. Polypeptides according to claim 1, 2 or 3 comprising immunoglobulin heavy and light chains capable of forming complete anti-Rhesus D antibodies.
- 6. DNA sequences coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V<sub>H</sub> and V<sub>L</sub> with the same or different identification numbers according to the figures given in the table below and functional equivalents thereof:

		\	/н		٧ <sub>L</sub>							
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.				
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288				
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288				
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285				
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285				
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288				
LD2-1	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294				
LD2-4	Fig: 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282				
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288				
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294				
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285				
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285				
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285				
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285				
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285				
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285				
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285				

7. DNA sequences according to claim 6 coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V<sub>H</sub> and V<sub>L</sub> with the same

identification numbers according to the figures given in claim 6, and functional equivalents thereof.

- 8. DNA sequences according to claim 6 or 7 which include regions with the DNA sequences  $V_{\rm H}$  and  $V_{\rm L}$  with the identification numbers according to the figures given in claim 6.
  - DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming antigen binding Fab fragments.
  - 10. DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming complete anti-Rhesus D antibodies.
- 11. A process for preparing recombinant polypeptides capable of forming antigen binding structures, e.g. Fab fragments, with specificity for Rhesus D antigens which process comprises the following steps in sequential order:
  - a) boosting of an individual capable of forming anti-Rhesus D antibodies with Rhesus D positive red blood cells,
  - b) isolating mononuclear cells from the individual,

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- c) isolating total RNA from the mononuclear cells,
- d) preparing a cDNA by using an oligo(dT)primer and reverse transcribing of the mRNA with M-MuLV reverse transcriptase and amplifying the cDNA repertoire by a polymerase chain reaction using immunoglobulin gene family specific primers,
- e) creating a phage display library by inserting the DNA coding for the heavy and light chain of the Fab polypeptide into a phagemid vector; the DNA for the heavy chain is inserted in frame to the gene coding for the phage protein pIII which allows the expression of a Fab pIII fusion protein on the surface of the phage,
- f) transforming bacterial cells with the obtained recombinant plasmids, cultivating of the transformed bacterial cells and co-expression of the heavy and the light chain of a Fab on filamentous phage particles,

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- g) amplifying the Fab-carrying phage in bacteria,
- h) selecting individual phage clones by several rounds of panning on Rhesus positive red blood cells.
- i) isolating the plasmid DNA from the selected clones and cutting out the gIII gene,
- j) transforming bacterial cells with the obtained plasmid, cultivating of the transformed bacterial cells expressing the Fab, and isolating the Fab fragments.
- 12. A process for selecting recombinant polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens and in particular showing reactivity with the Partial Rhesus DVI Variant and without any evidence of reactivity with red blood cells of Rhesus negative phenotypes in particular without reactivity against the Rhesus alleles C, c, E, and e which process comprises the following steps in sequential order:
- a) performing several negative absorptions on the following red blood cells: phenotype 1 (r'r, Ccddee) treated with bromelase, phenotype 1 not treated with bromelase, phenotype 2 (ryry, CCddEE) treated with bromelase and phenotype 2 not treated with bromelase,
- b) performing a positive absorption on DVI+ red blood cells with or without bromelase treatment,
  - c) determining the titer of phage binding to DVI+ red blood cells
  - d) repeating steps a), b) and c) until the titer of phage binding to DVI+ red blood cells has reached a satisfactory level.

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- 13. A process according to claim 12, wherein the recombinant polypeptides capable of forming antigen binding structures are Fab fragments.
- 14. Anti-Rhesus D antibodies having heavy and light chain variable
   regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3

sequences of pairs of amino acid sequences  $V_{\text{H}}$  and  $V_{\text{L}}$  having the same or different identification numbers according to the table below:

			/н	V <sub>L</sub>							
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.			
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288			
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288			
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285			
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285			
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288			
LD2-I	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294			
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282			
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288			
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294			
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285			
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285			
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285			
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285			
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285			
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285			
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285			

- 15. Anti-Rhesus D antibodies having heavy and light chain variable regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3
  sequences of pairs of amino acid sequences V<sub>H</sub> and V<sub>L</sub> having the same identification numbers as indicated in the table of claim 14.
  - 16. Anti-Rhesus D antibodies according to claim 14 or 15 which include pairs of amino acid sequences  $V_H$  and  $V_L$  having the identification numbers according to the figures, as indicated in the table of claim 14.
- 17. Anti-Rhesus D antibodies according to claims 14, 15, or 16 wherein the immunoglobulin constant regions are of at least one of the defined isotypes IgG1, IgG2, IgG3 or IgG4.

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- 18. A process for preparing complete anti-Rhesus D antibodies according to one of the claims 14 to 17, comprising in sequential order the steps of
  - a) amplifying separately the members of a pair of a heavy chain V gene segment and a light chain V gene segment containing Rhesus Dspecific CDR 1, CDR 2 and CDR 3 regions as depicted in Figs. 1a -16a and 1b - 16b, respectively, from an anti-Rhesus D-Fab-encoding plasmid by carrying out a polymerase chain reaction with specific primers,
- b) preparing separately the genes of a complete anti-Rhesus D immunoglobulin heavy chain and a complete anti-Rhesus D immunoglobulin light chain in suitable plasmids containing the immunoglobulin constant region gene segments coding for either one of the human γ1, γ2, γ3 and γ4 heavy chains and for the human κ or λ light chain and transforming the obtained plasmids separately in suitable E. coli bacteria, and
  - c) cotransfecting the obtained plasmids into suitable eukaryotic host cells, cultivating of the cells, separating the non-transformed cells, cloning of the cultures, selecting the best producing clone, using it as a production culture and isolating the complete antibodies from the supernatant of the cell culture.
  - 19. A pharmaceutical composition comprising at least one polypeptide according to the definition of claim 1, 2 or 3 or at least one anti-Rhesus D antibody according to one of the claims 14 to 17 for the prophylaxis of haemolytic disease of the newborn, for the treatment of idiopathic thrombocytopenic purpura and mistransfusions of Rhesus incompatible blood.
    - 20. A diagnostic composition for Rhesus D typing comprising Fab fragments according to claim 4 or anti-Rhesus D antibodies according to one of the claims 14 to 17.

# Fig. 1a

## LD1-40-VH sequence

			9			18												54
5'	CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	AGG	TCC	CTG
	Q	v	К	L	L	E	S	G	G	G	v	v	Q	P	G	R	s	L
			63			72			81			90			99			108
	AGA	CTC	TCC	TGT	ATA	GCG	TCT	GGA	TTC	ACC	CTC	AGG	AAT	TAT	GCC	ATG	CAC	TGG
	 R	L	s	C	I	A	s	G	F	T	L	R	N	Y	A	М	Н	W
													←		- CDF	RI —	<del></del>	
			117			126			135			144		- c-m	153	mcc	man	162
	GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GCA	GGT	ATA	166		GA!
	v	 R	Q	 А	P	G	к	G	L	E	W	· v	Α	G	I	W	£	Ď
														←—		CDR2		
			171	•		180	:		189	· 		198	~~~	mmc	207	3 m.~	mac	216
	GGA	AGT	AAC	AAA	AAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA		ACC	AIC		
	G	S	N	К	N	Y	Α	D	Ş	V	К	G	R	F	T	I	S	R
			225			- CDF	12		243			252			261			270
, '	GAC	AAT	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	CTG	AAC	AGC					GAC
	D	N	s	к	N								s			D	Ε	D
			279			288			297		-	306			315			324
٠.	ACG	GCT	GTG	TAT	TAT	TGT	GCG	AGA	GAG	CGA	GCA	GCA	CGT	GGT	ATT	TCT	AGG	TTC
	T	A	v	Y	Y	c	 A						R			s	R	F
			222			242							- CD					
	TAT	TAC	333 TAC			GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	CCA	3'
											 T	 T			 V		P	
	Y	Y		M				G	ĸ	G	1	1	•	٠	•	_	•	
			(	CDR3			7											

# Fig. 1b

# LD1-40-VL sequence

	9			18			27			36			45			54
ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGC	GAC	AGA	GTC	ACC
M	T	Q	s	P	s	s	L	s	A	s	v	G	D	R	v	T
	63			72	<b>616</b>	1.00	81	1.00	N.C.C	90 CAT	<b>ው</b> ሞር	חתת	99 TGG	ጥልጥ	CAG	108
ACT	TGC		GCA	AGT	CAG	AGC	A11	AGG	AGC							
T	С	R	Α	s	Q	s	I	R	s	Н	L	И	W	Y	Q	Q
		<del></del>					CDR	l —				<del>&gt;</del>				
	117			126			135			144			153	mm.c	~ n n	102
CCA	GGG	AAA	GCC	CCT	AAG	TTG	CTG	ATC	TAT	GGT	GCG	TCC	ACT	TTG	CAA	AGI
					к	 L	L	I	Y	G	Α	S	T	L	Q	5
-	-	••	• •	_						<del></del>			CDR2			<del>&gt;</del>
	171			180			189						-			216
GTC	CCA	TCA	AGG	TTC	AGT	GGC	AGT	GGC	TCT	GGG	GCA	GTT	TTC	ACT	CTC	ACC
													 F	т	T.	T
V	P	5	К	r	٥	G	3	G	د	G	Α.	•	٠	•	~	-
	225			234			243		•	252			261			270
	AGT	CTA	CAA	CCT	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	GAG	AGT	TAC
	 s	L	Q	- <del>-</del> -	 E	 D	 F	Α	T	Υ	Y	c	Q	E	s	Y
							207			206			₹15			
AAT		СТА	ATC	ACC	TTC	GGC	CAA	GGG	ACA	CGA	CTG	GAG		AAA	3'	
N	P		I	 T	 F	 G	Q	G	T	R	L	E	т	к		
	M ACT T CCA P GTC V GCC A AAT	ATG ACC  M T  63  ACT TGC  T C  117  CCA GGG  P G  171  GTC CCA  V P  225  GCC AGT  A S  279  AAT CCT	ATG ACC CAG  M T Q  63 ACT TGC CGG  T C R  117 CCA GGG AAA  P G K  171 GTC CCA TCA  V P S  225 GCC AGT CTA  A S L  279 AAT CCT CTA	ATG ACC CAG TCT  M T Q S  ACT TGC CGG GCA  T C R A  CCA GGG AAA GCC  P G K A   117  GTC CCA TCA AGG  V P S R  GCC AGT CTA CAA  A S L Q  AAT CCT CTA ATC	ATG ACC CAG TCT CCA  M T Q S P  63 72 ACT TGC CGG GCA AGT  T C R A S  CCA GGG AAA GCC CCT  P G K A P  171 CCA AGG TTC  V P S R F  GCC AGT CTA CAA CCT  A S L Q P  AAT CCT CTA ATC ACC	ATG ACC CAG TCT CCA TCC  M T Q S P S  ACT TGC CGG GCA AGT CAG  T C R A S Q  CCA GGG AAA GCC CCT AAG  P G K A P K  GTC CCA TCA AGG TTC AGT  V P S R F S  GCC AGT CTA CAA CCT GAA  A S L Q P E  279  AAT CCT CTA ATC ACC TTC	ATG ACC CAG TCT CCA TCC TCC  M T Q S P S S  ACT TGC CGG GCA AGT CAG AGC  T C R A S Q S  CCA GGG AAA GCC CCT AAG TTG  P G K A P K L  GTC CCA TCA AGG TTC AGT GGC  V P S R F S G  GCC AGT CTA CAA CCT GAA GAT  A S L Q P E D  AAT CCT CTA ATC ACC TTC GGC	ATG ACC CAG TCT CCA TCC TCC CTG  M T Q S P S S L  63 72 81  ACT TGC CGG GCA AGT CAG AGC ATT  T C R A S Q S I  CDR: 117 126 CTG AAG TTG CTG  P G K A P K L L  171 CCA AGG TTC AGT GGC AGT  V P S R F S G S  GCC AGT CTA CAA CCT GAA GAT TTT  A S L Q P E D F  279  AAT CCT CTA ATC ACC TTC GGC CAA	ATG ACC CAG TCT CCA TCC TCC CTG TCT  M T Q S P S S L S  ACT TGC CGG GCA AGT CAG AGC ATT AGG  T C R A S Q S I R  CDR1  117  CCA GGG AAA GCC CCT AAG TTG CTG ATC  P G K A P K L L I  GTC CCA TCA AGG TTC AGT GGC AGT GGC  V P S R F S G S G  GCC AGT CTA CAA CCT GAA GAT TTT GCA  A S L Q P E D F A  279  AAT CCT CTA ATC ACC TTC GGC CAA GGG	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA  M T Q S P S S L S A  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC  T C R A S Q S I R S  CDR1  117  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT  P G K A P K L L I Y  GTC CCA TCA AGG TTC AGT GGC AGT GGC TCT  V P S R F S G S G S  GCC AGT CTA CAA CCT GAA GAT TTT GCA ACT  A S L Q P E D F A T  279  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT  M T Q S P S S L S A S  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT  T C R A S Q S I R S H  CDR1  117  126  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT  P G K A P K L L I Y G  171  GTC CCA TCA AGG TTC AGT GGC AGT GGC TCT GGG  V P S R F S G S G S G  GCC AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC  A S L Q P E D F A T Y  279  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA  M T Q S P S S L S A S V  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT TTG  T C R A S Q S I R S H L  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT GCG  P G K A P K L L I Y G A  171 180 180 189 198  GTC CCA TCA AGG TTC AGT GGC AGT GGC TCT GGG GCA  V P S R F S G S G S G A  GCC AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC TAC  A S L Q P E D F A T Y Y  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGC  M T Q S P S S L S A S V G  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT TTG AAT  T C R A S Q S I R S H L N  CDR1  117  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT GCG TCC  P G K A P K L L I Y G A S  TT C CA AGG TTC AGT GGC AGT GGC TCT GGG GCA GTT  V P S R F S G S G S G A V  CCA AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC TGT  A S L Q P E D F A T Y Y C  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG	ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGC GAC  M T Q S P S S L S A S V G D  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT TTG AAT TGG  T C R A S Q S I R S H L N W  CDR1  117  126  135  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT GCG TCC ACT  P G K A P K L L I Y G A S T  CDR2  171  GTC CCA TCA AGG TTC AGT GGC AGT GGC TCT GGG GCA GTT TTC  V P S R F S G S G S G A V F  GCC AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA  A S L Q P E D F A T Y Y C Q  279  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGC GAC AGA  M T Q S P S S L S A S V G D R  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT TTG AAT TGG TAT  T C R A S Q S I R S H L N W Y  CDR1  117  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT GCG TCC ACT TTG  P G K A P K L L I Y G A S T L  CTR CCA TCA AGG TTC AGT GGC AGT GGC TCT GGG GCA GTT TTC ACT  OV P S R F S G S G S G A V F T  CCA GGG AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA GAG  A S L Q P E D F A T Y Y C Q E  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGC GAC AGA GTC  M T Q S P S S L S A S V G D R V  ACT TGC CGG GCA AGT CAG AGC ATT AGG AGC CAT TTG AAT TGG TAT CAG  T C R A S Q S I R S H L N W Y Q  CDR1  CCA GGG AAA GCC CCT AAG TTG CTG ATC TAT GGT GCG TCC ACT TTG CAA  P G K A P K L L I Y G A S T L Q  T111  180  189  198  198  198  207  GTC CCA TCA AGG TTC AGT GGC AGT GGC TCT GGG GCA GTT TTC ACT CTC  V P S R F S G S G S G S G A V F T L  CCC AGT CTA CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA GAG AGT  A S L Q P E D F A T Y Y C Q E S  AAT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA 3'  A S C C CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA 3'  AT CCT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA 3'

# Fig. 2a

## LD1-52-VH sequence

		9			18			27			36			45			5
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG 	TCC	CT
Q	v	ĸ	L	L	E	s	G	G	G	V	٧	Q	P	G	G	s	. T
		63			72			81			90			99			10
AGA	CTC		TGT	GAA		TCT	GGA	TTC	GCC	CTC	AGA	AGT	TCT	GGC	ATG	CAC	TG
 R	 L	 S		Ξ	 А	s	 G	£	Α	L	R	S	s	G	M	Н	W
									-			<del></del>		CDRI			
GTC	CGC	117 CAG	GCT	ССТ	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	GCA	СТТ	ATA	TGG		
	 R	0	 A	 P		~	 G			w		Α	L L	I	w	F	D
		-											<del></del>		CDR2		21
		171			180			189	cmc	220	198	CCA	<b>תתר</b>	207	אדר	TCC	21 AG
GGA	AGT	ATC	AGA	TCG	TAT	GCA	GAA	TCC	GTG	AAG							
G	s	I	R	S	Y	Α	E	s	v	K	G ·	R	F	T	I	S	R
					- CDF	₹2						•		261			27
		225			234	CE B	TAT	243	כאא	ስጥC	252 CGC	AGT	CTG		GCC	GAC	
GAC	ACT	TCC	AAG	AAC	ACC	CIA											
D	T	S	K	N	T	L	Y	L	Q	M	R	\$	L	S	Α	D	D
		279			288			297			306		٠.	315			32
ACG	GCT	GTG	TAT	TAC	TGT	GCG	AGA	GAC	AAG	GCG	GTT	CGG	GGA	ATT	AGC	AGG	TA
											v						 Y
T	Α	٧	Y	Y	С	A	R	D									
		333			342			351			360			369			
AAC	TAT	TAC	ATG	GAC	GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	TCA	3
N		 Y	 M	D	v	W	G	К	G	T	T	V	T	٧	5	s	
		CDR3				<b>→</b>											

#### Fig. 2b

#### LD1-52-VL sequence

		9			18			27			36			45			5
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	AC
 v		 T	Q	s	P	S	s	L	s	A	s	v	G	D	R	v	T
ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AAC	81 ATT	ATC	CGC	90 TAT	TTA	AAT	99 TGG	TAT	CAG	10 CA
	 T		 R	 A	s	Q	N	I,	I	R	Y	L	N	W	Y	Q	Ç
			<del></del>					CDR					<del></del>				
AAG	CCA	117 GGG	AAA	GCC	126 CCT	AGG	CTC	135 CTG	ATC	TAT	144 GGT		TCC	153 ACT	TTG	CAA	16 A0
	P	 G	к	Α	P	R	L	L	I	Y	G	A	S	T	L R2 —	Q	
								189			198			- CD 207	K2 —		21
GGG	GTC	171 CCA	TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT			GAT		ACT	CTC	AC
 G	 V	 P			- <u>-</u> -			<b></b> S,	- <b></b> G	s	 G	T	D	F	T	L	7
ATC	AGT	225 AGT	CTG	CAA	234 CCT	gaa	GAT	243 TTT	GCA	ACT	252 TAC		TGT	261 CAA	CAG	AGT	27 T/
	 S	 s		Q	 P		D	F	Α	T	Υ	Y	С	Q	Q	s	-,
ccm	אכר	279	CCA	TTC	288 ACT	<del>ተ</del> ሞሮ	<b>G</b> GC	297 CCT	GGG	ACC	306 AAA		GAG	315 ATC		3'	
	ACC																
R	Т	P	P	F	T	F	G	P.	Ģ	T	K	V	E	1	K		
		- CDF	₹3 —			•											

# Fig. 3a

#### LD1-84-VH sequence

		9			18			27			36			45			5
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CT
Q	v	к	L L	L	E	s	G	G	G	V	v	Q	P	G	G	S	L
AGA	CTC	63 TCC	TGT	GAA	72 GCG	тст	GGA	81 TTC	ACC	стс	90 AGA	AGT	TCT	99 GGC	ATG	CAC	10 TG
 R		s	С	 Е	Α	s	G		T	L	R	s	s	G CDR1	M	H	W
GTC	CGC	117 CAG	GCT	ССТ	126 GGC	AAG	GGG	CTG	GAG	TGG	144 GTG			153			16
 V	 R	Q	 A	P		к	G	L		W	V		L	I	W W	F	Ε
GGA	AGT	171 ATC	AGA			GCA	GAA	TCC	GTG	AAG		CGA	TTC	207 ACC	ATC	TCC	21 AC
G	s	I	-								G	R	F	Т	I	S	F
GAC	ACT	225 TCC			224	CTA		243 CTC	CAA		252 CGC	AGT	CTG	261 AGT	GCC	GAC	27 G/
D	 T	s	к	N	T	L	Y		Q	М	R	S	L	s	A	D	1
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGC	AGG	32 T/
T	A	v	Y	Y	c	Α	R	D	ĸ	Α	٧	R	G TOD3	<u> </u>		R	
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351			360			369		TCA	. :
N	 Y	Y		D	v	W	G	К	G	T	Т	v	T	v	S	S	
		— CD	R3 -			→											

## Fig. 3b

#### LD1-84-VL sequence

GTG	ATG	9 ACC	CAG	тст	18 CCA	TCC	TCC	27 CTG	TCT	GCA		ATA		45 GAC	AGA	GTC	54 AC
				 s		 s			 S	 A	 S	 I		 D	 R	 V	 T
V	М	1	Q	3	_							_	-	•			10
NTC.	אככ	63 TGC	cee	GCA	72 ACT	CAG	AGT	81 ATC	ATC	AGG	90 TAT	TTG	AAT	99 TGG	TAT	CAG	10 CA
I	Ť	С	R	Α	S						Y	L	N.	W	Y	Q	
		117	←		126			— CE			144			153			16
AAA	CCA	GGA	AAA	GCC	CCT	AAA	СТС	CTC	ATC	TTT	GCT		TCG	AAT	TTG	CAA	AC
													 S			0	
ĸ	P	G	К	A	Р	K	ь	ь	1	£	<u> </u>		_	CDR2		<u> </u>	
		171			180			189			198			207			21
GGG	GTC	CCA	TCC	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGG	ACA	GAT	TTC	ACT	CTC	AC
 G	v	P	<b>-</b>	R	 F	s	G	s	G	s	G	T	D	F	T	L	Т
		225			234			243			252			261			27
ATC	AGT	GAC	CTG	CAG	CCT	GAG	GAT	TTC	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TA
	 s	D	 L	 Q	 P	E	D	F	Α	т	Y	Y	С	Q	Q	s	Y
		279			288			297			306			<del>←</del> 315			
AGT	AGG	CCG	TTC	ACT	TTT			GGG	ACC	AGC	CTG		ATC	AAA	3'		
 S	 R	 P	 F	т	 F	 G	 R	 G	т	 S	L	D	I	к			
-		_		<del>&gt;</del>													

#### Fig. 4a

#### LD1-110-VH sequence

			9			18			27			36			45			54
5'	CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	AGG	TCC	CTG
		 V	 К			 E	s	G	G	G	v	v	Q	P	G	$\mathbf{R}_{j}$	s	L
	AGA	CTC	63 TCC	TGT	ATA	72 GCG	TCT	GGA	81 TTC	ACC	стс	90 AGG	AAT	TAT	99 GCC	ATG	CAC	108 TGG
	 R	 L	 S				 S	G	F	T	L	R	N				Н	W
	GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	CTG		TGG 		GCA	GGT		TGG		162 GAT
	v	R	Q	A	P	G	ĸ	G	L	E	W	V	Α	G ←—	I	W - CD!	F 22 —	D
	GGA	AGC	17:1 AAC	ааа	AAC	180 TAT	GCA	GAC	189 TCC	GTG	AAG	198 GGC	CGA		207			216 AGA
	G	s	N	K	N	Y	A	D	s	V	K	G	R	F	Т	I	S	R
	GAC	AAC	225 TCC	AAG		224	CTG		213			252 AAC	AGC	CTG	261 AGA	GCC	GAG	270 GAC
	D	N	s	K	N	Т	L	F	L	Н	M	N	s	L	R	A	E	D
	ACG	GCT	279 ACA	TAT	TAC	288 TGT	GCG	AGA	GAG	AGG	GCG	306 ATT	CGG	GGA	315 ATC		AGA	324 TAC
	 T	 A	 T	 Y	 Y	c	Α	R	E	R	Α	I	R	G			R	Y
	AAT	TAC	333 TAC	ATG	GAC	342 GTC	TGG	GGC	251			360	GTC		369			. 3'
	N	Y	Y CDR3	м 3	D	v	w →	G	к	G	T	T	V	Т	V	S	S	

#### Fig. 4b

#### LD1-110-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v	M	T	Q	 s	P	s	s	L	s	A	s	v	G	D	R	V	T
		63			72			81			90	<b></b>		99		c».c	
ATC	ACT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	CGA	AGC	TCT	TTA	AAT	TGG	TAT	CAG	CAG
I	T	С	R	A	s	Q	s	I	R	s	s	L	N	W	Y	Q	Q
								CDR	l —				<del></del>				
		117			126			135			144						
AAA	CCA	GGG	AAA	GCC	CCT	AAA	GTC	CTG	ATC	TAT	GCT	GCA	TCC	AGT	TTG	CAA	AGT
ĸ	P	G	ĸ	A	P	K	v	L	I	Y				s			S
														- CD			
		171			180			189			198		~~~	207	3 CM	cmc	216
GGG	GTC	CCA	TCC	AGG	TTC	AGT	GGC	AGA	GGA	TCT	GGG	ACA	GAT	TTC	ACT		
G	v	P	s	R	F	S	G	R	G	S	G	T	D	F	T	L	Ť
		225			234			243			252			261			270
ATC	AGC	AGT	CTG	CAG	CCT	GAA	GAT	TTT	GCG	ACT	TAT	TAT	TGT	CAA	CAG	AGT	TCC
I	s	 s	L L	Q	P		D	F	Α	T	Υ	Y	С	Q	Q	s	s
		270			288			297			306			315			
AGT	TCC	TCG	TGG	ACG	TTC	GGC	CAA	GGG	ACC	AAG	GTG	GAA	ATC	AAA	3'		
s	 s	s	W	Ţ	F	G	Q	G	T	ĸ	v	E	I	K			
	— cг	R3 -		<del></del>													

#### Fig. 5a

#### LD1-117-VH sequence

,		cmc	9	CTG	CTC	18	тсъ	GGA	27 GGA	GGC	GTG	36 GTC	CAG	CCT	45 GGG	AAG	TCC	54 CTG
	CAG	GTG																
	Q	v	K	L	L	E	s	G	G	G	V	v	Q	P	G	K	S	L
			63			72			81									108
	AGA	CTT	TCC	TGT	GCA	GCG	TCT	GGA	TTC	AGT	TTC	AAT	AGC	CAT	GGC	ATG	CAC	TGG
		 t.	 S	 С			 s		 F	s	F	N	s	Н	G	M	H	W
			_				-								CDR1			
			117			126			135			144			153			162
	GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GCA	TTT	ATA	TGG	TTT	GAT
																		D
	V	R	Q	Α	5	G	ĸ	G	L	Е	W	V	Α	r		~~~~	_	_
															207	CDRZ		216
			171			180			189		220	198	CCA			ልጥር	ACC	
	GGC	AGT	AAT	AAA	TAC	TAT	GCA	GAC	TCC	GTG	AAG							
	 G	 S	N	ĸ	Υ	Y	 A	D	s	v	ĸ	G	R	F	T	I	T	R
						— ci	DR2 -											
			225			234			243			252						270
	GAC	AAC	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	AGC	CTG	AGA	GCC	GAG	GAC
	D	и 	 s	 К	N	T	L L	Y	L	Q	М	N	s	L	R	A	E	D
						200			297			306			315			324
	N.C.C	CCT	279	TAT	ጥልሮ	<b>76</b> 0	GCG	AGA	GAG	ACC	TCA	GTA	AGG	CTA	GGG	TAT	AGC	CGC
	T	Α	v	Y	Y	С	Α	R	E	T	S	V	R	L	G	Y	S	R
									<del></del>			2.50	— СБ	кз —	369			378
	TAC	AAT	333 TAC	TAC	ATG	342 GAC	GTC	TGG	351 GGC	AAA	GGG	ACC	ACG	GTC	ACC	ATC	TCG	
	 Y	 N	 Y	 Y	 M		 V	 W	 G	к	G	T	T	v	Ť	I	s	S
	-	• •	-	-	13 <del></del>													

#### Fig. 5b

## LD1-117-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v		T	Q	s	P	s	s	L	s	A	s	v	G	D	R	v	T
		63			72			81			90			99			108
ATC	ACT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	AGG	AGC	CAT	TTG	AAT	TGG	TAT	CAG	CAC
· I	T	C -	R	A	S	Q	S	I	R	S	H	L	N	W	Y	Q	Q
								CE	R1 -				<del></del>	·			
		117			126			135			144			153	mm.c	~ n n	163
AAA	CCA	GGG	AAA	GCC	CCT	AAG	CTC	CTG	ATC	TAT	GCT	GCA	TCC	AGT	TTG	CAA	GG !
														s	1.	0	G
K	P	G	К	A	P	K	r	1	÷					CDR2		_	
					100			100			•						216
GGG	GTC	171 CCA	TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGG	ACA	GAT				
G	V	P	S	R	F	S	G	S	G	S	G	Т	D	F	T	L	T
		225			234			243			252			261			270
ATC	AGC.	AGT	CTG	CAA	CCT	GAA	GAT	TTT	GCA	ACT	TAT	TAC	TGT	CAA	CAG	AGT	TAC
												 Y			0		
I	S	S	L	Q	P	Ŀ	D	F	М	•	. •	•	•	<u>×</u>	¥		
		279			288			297			306			315		-	
AGG	GCC	CCT	CAG	TGG	ACG	TTC	GGC	CAA	GGG	ACC	AAG	GTG	GAA	ATC	AAA	3 '	
- <b></b> R	 A	 P	0	 W	 T		 G	Q	G	т	K	v	E	I	к		
		_	~					_									
		CDI			•												

#### Fig. 6a

#### LD2-1-VH sequence

CAG	GTG.	9 444	CTG	стс	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG					54 CTG
																 S	 L
Q	V	K	L	L	E	S	G	G	G	V	V	Q	P	G	G	5	ы
		63			72			81			90			99			108
AGA	CTC	TCC	TGT	GTA	GCG	TCT	GGA	TTC	ACC	CTC	AGG	AGT	TAT	GGC	ATG	CAC	TGG
 R	L	s	С	v	A	s	G	F	T	L	Ŗ	s	Y	G	M	н	W
																	<b>→</b>
<u> </u>		117			126			135	C D C	mcc	144	CCT			тсс		
GTC	CGC	CAG	GCT	CCA	GGC	AAG		CTG	GAG	100							
v	R	Q	Α	P	G	K	G	L	E	W	v	Α					D
											100		-	207	CDR2		216
CCA	AGT	171	מממ	CCA	180	GTA.	GAC	189	стс	AAG	GGC	CGA	TTC		АТС	TCC	
GGA																	
G	S	N	к							ĸ	G	R	F	T	I	S	R
					- CDF	12 —		242			252			261			270
GAC	AAT	225 TCC	AAG	AAC	ATG	GTC	TAT	CTG	CAA	ATG	AAC		CTG		GCC	GAT	
						 v	 Y	 L	 Q	 M			 L				 D
D	N	S	K	N	M	V	1	T	Q	М	14	-	-	••	••	-	_
		279			288			297			306			315			324
ACG	GCT	GTA	TAT	TAT	TGT	GCG	AGA	GAG	AAG	GCG	CTT	CGG	GGA	ATC	AGC	AGA	TAC
T	Α	v	Y	Y	С	A					L				S		Y
								<del></del>				- CD	R3				
אאר	TAT	333 TAC	ርፕር	GAC	342 GTC	TGG	GGC	351 AAG	GGG	ACC	360 ACG		ACC	369 GTC	TCC	TCA	31
N	Y	Y		D	V	W	G	ĸ	G	T	T	V	T	V	S	S	
		CDR3			<del></del>												

#### Fig. 6b

#### LD2-1-VL sequence

		9			18			27			36			45			54
GTG	GTG	ACT	CAG	CCA	CCC	TCA	GCG	TCT	GGG	ACC	CCC	GGA	CAG	AGG	GTC	ACC	ATC
 V		 T	Q	P	P	s	Α	s	G	т	Р	G	Q	R	v	T	I
TCT	TGT		GGA	AGC	72 AAC	TCC	ATC	81 CTT	GGA	AGT	90 AAG	TAT	GTA	99 TAC	TGG	TAC	108 CAG
 s			G			s		L			к	Y	V	Y	W	Y	Q
ааа	стс		GGA		100		— с ааа	125		ATC	144 TAT	AAG	AAT	153 GAT	CAG	CGG	162 CCC
 К	 L	P	G .	 T	Α	P	К	L	L		Y	K	N 	D .	Q CDR2	R	P
TCA	GGG	171 GTC	TCT	GAC	180 CGA	TTC	TCT	189 GGC	TCC	AAG	198 TCT			207			216
 s	 G	v	s	 Э	R	F	s	G	s	K	S	G	T	S	A	s	L
—→ GCC	ATC	225 AGT	GGG	cTC	234 CGG	TCC	GAG	243 GAT	GAG	GCT	252 GAC	TAT	TAC	261 TGT		CCA	270 TG0
Α	I	s	G		R	s	E	D	E		D	Y	Y	С	A ←	P	W
GAT	GCC		CTG	GGT	288 GGC	CCG	GTG	297 TTC	GGC	: GGA	306 . GGG	ACC	AAG	315 CTG	ACC	GTC	32 cT/
D		N	L Ci						G	G	G	Т	K	L	T	٧	L
AGT		333 CCC	; 3'														
s	. <u></u> -	P	•														

#### Fig. 7a

#### LD2-4-VH sequence

		9			18			27				a		45	ccc	mcc.	54
CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	GTG	GTC	CAG				TCC	
Q	v	К	L	L	E	S	G	G	G	V	V	Q	P	G	G	S	L
AGA	CTC	63 TCC	TGT	GÁA	72 GCG	TCT	GGA	81 TTC	ACC	CTC		AGT				CAC	108 TGG
 R		 s		 Е		s	G	F	т	L	R	s	s	G	M	H	W
						2										<del></del>	
GTC	CGC	117 CAG	GCT	ССТ	126 GGC	AAG	GGG	135 CTG	GAG	TGG	GTG	GCA	CTT	153 ATA	TGG	TTT	162 GAT
 V	 R	<b></b> Q	 A	 P	 G	к	 G	L	E	W	v	A		I	W	F	D
													<del></del>		CDR2		216
GGA	AGT	171 ATC	AGA	TCG	180 TAT	GCA	GAA	189 TCC	GTG	AAG	198 GGC	CGA	TTC	207 ACC	ATC	TCC	
 G	 s	ī	R	s	Y	A	E	s	v	ĸ	G	R	F	T	I	s	R
						2	<del>-</del>							261			270
GAC	ACT	225 TCC	AAG	AAC	234 ACC	CTA	TAT	243 CTC	CAA	ATG	252 CGC	AGT	CTG		GCC	GAC	_
		 s		 N	 Т	 L	·Y		Q	М	R	s	L	s	A	D	D
ACG	GCT	279 <b>GT</b> G	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT		AGG	324 TAC
 Т	Α	v	Y	Y	c	Α	R			A			G				Y
								<del></del>				-, CD	R3 —				
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA	.GGG	ACC	360 ACG		ACC	369 GTC	TCC	TCA	3'
N	Y	Υ	M	D	v	W	G	ĸ	G	Т	T	v	T	v	s	s	
		- CD	R3 —			<b>→</b>											

## Fig. 7b

#### LD2-4-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v	 М	т	Q	5	P	s	s	L	s	A	S	v	G	D	R	V	T
አጥር	እርጥ	63 TGC	cee	DCD.	72 AGT	CAG	ACC	81 ATT	AGC	AGA	90 AAT	TTA	AAT	99 TGG		CAG	108 CAG
 I	лот 	 C	 R			 Q		 I		 R	 N	 L	 И	 W	 Y	 Q	 Q
1	1	C	,			_		_									
AAA	CCA	117 GGG	AAA	GCC	126			135			144 GCT	ACA	TCC	153 AGT	TTG	CAA	162 AGT
	 P											T	s	s	L	Q	s
											<del></del>			CDR2			—→ 216
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	тст	198 GGG		GAT	207 TTC	ACT	СТС	
 G	 V	 P	 s	 R	 F	s	 G	s	 G	s	G	T	D	F	T	L	T
አጥሮ	አአጥ	225 ACT	СТА	۵۵ م	234 CCT	CAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
ATC	AAI	AGI															
1	N	S	L	Q	P	E	D	F	Α	T	Y	Y	С	Q	Q	S	Y
	100	279	mcc.	mm.c	288	<b>C N N</b>	GGG	297	AAG	GTG.	306 GAA	ATC		315			_
ACT	ACC	CCT	100														
T	T	P	s	F	G	Q	G	T	K	٧	E	I	K				
	– CD	R3 —	<del></del>														

#### Fig. 8a

#### LD2-5-VH sequence

		9			18			27						45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	TTG	GTC	CAG	CCG	GGG 	GGG	TCC	CTG
Q	v	ĸ	L	L	E	s	G	G	G	L	V	Q	P	G	G	S	L
	CTC	63		am.	72	m.cm	CCA	81	. מככ	<b>ጥጥ</b> ር	90 AGG	аст	ТАТ	99 GGC	ATG	CAC	108 TGG
AGA	CTC	TCC	TGT	GTA	GLG	TCT	.GGA		ACC								
R	L	S	С	v	Α	s	G	F	T	F	R	S	Y	G	М	••	W
••	_	_										<del></del>		CDR1			
		117			126			135			144			153			
GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGC	CTG	GAG	TGG	GTG	GCT	TTT	ATA	TGG		GAT
				 P						 W				I	W	F	D
V	R	Q	А	P											CDR2		
		171			180			189			198			207			216
GGI	AGT	TAAT	AAA	GGA	TAT	GTA	GAC	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	CGA
						~											
G	S	N	K	G	Y	V	D	S	V	K	G	R	F	Т	Ţ	S	К
			<u> </u>	(							<del></del>			261			270
		225			234			243	~~ ~	N TO C	252	אכר	ርፕር		GCC	GAG	
GAC	TAA	TCC	AAG	AAC	ATG	CTC	TAT	CTG	CAA	A1G	~~.						
D	N	S	ĸ	N	М	L	Y	L	Q	M	N	S	L	R	A	E	D
		220			200			297			306			315			324
אכנ	GCT	279	тат	ጥልጥ	200 ጥርጥ	GCG	AGA	GAG	AAG	GCG	CTT	ÇGG	GGA	ATC	AGT	AGA	TAC
ACC	, GC1	917															
т	А	V	Y	Y	С	Α	R	E	ĸ	Α	L	R	G	I	S	R	Y
								-				— CD	R3 —				
		333			342			351			360	cmc	N.C.C	369	<b>תרר</b>	TCD	31
AA	TAT	TAC	CTG	GAC	GTC	TGG	GGC	AAG	GGG	GCC	ACG	910					-
N	Y	 Y	L	D		W	G	K	G	A	Т	٧	T	V	S	s	
		— CD	R3 -			<b>→</b>											

#### Fig. 8b

#### LD2-5-VL sequence

			9			18			27			36			45			54
,	GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	GGC	GAC	AGA	GTC	ACC
	 V	 M	 T	 Q	 S	P			L					G	D	R	V	T
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 GTT	ĄCC	AGG	90 TCT	TTA	AAT		TAT		108 CAG
	 I	- <del>-</del> -		R	Α				V	T			L	N	W	Y	Q	Q
	AAA	CCA	117 GGG	AAA	GCC				CDRI 135 CTA			1 4 4		TCC	153 ACT	Т <b>Т</b> G 	CAA	
	К	- <b>-</b> -	G	K	A	P	R	L	L	I	Ē	A	Α		T - CD	L L	Q	S
	GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198 GGG	ACA		207	ACC	CTC	
	G	v	Р	S	R	F	S	G	s	G	S	G	T	D	F	Т	L	T
	ATC	AGC	225 AGT	СТG	CAA	234 CCT	GAG	GAT	243 TTT	GGA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AAT	270 TAC
		 S		L L	Q	P	E				_		Y			_	N	Y
	AGG	ACC	279	CAG	TGG	288 ACG	TTC	GGC	297 CAA	GGG	ACC	306 AAG	GTA	GAĄ	315 ATC		3'	
	 R		P		 W	т	F	G			Т							
			(	DR3			<b>→</b>											

#### Fig. 9a

#### LD2-10-VH sequence

CAG	GTG	9 AAA	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG
		 К			 Е	s		G					P	G	G	s	L
AGA	CTC	63 TCC	TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	CTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	108 TGG
								F			R		Y	G CDR1	M	H	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	135 CTG	GAG	TGG	144 GTG		TTT	153			162
 V	 R	Q	 A	P	G	ĸ	G	L	E			Α	F	I	W CDB2	F	D
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	TCC	GTG	AAG	198 GGC			207			216
 G		 N	 К					s			G	R	F	T	I	S	R
GAC	AAT	225 TCC	AAG	AAC	234 ATG	GTC	TAT	243 CTG			252 AAC	AGC	CTG	261 AGA	GCC	GAT	270 GAC
	 N	 S		И	M	v	Y	L	Q	М	N	S	L	R	A	D	D
					000			297 AGA		AAG	306 GCG			315 GGA		AGC	324 AGA
 Т								R	 E	К	Α	L	R	G	I	s	R
TAC									<del></del>	GGG	360	ACG	- CDF	ાં૩ 369			TCA
Υ	N	Y			D DR3 -		W	. G	К	G	T →	T	v	T	V	S	S

#### Fig. 9b

#### LD2-10-VL sequence

			9			18			27			36			45			54
5'	GTG	GTG	ACT	CAG	GAG	CCC	TCA	CTG	ACT	GTG	TCC	CCA	GGA	GGG	ACA	GTC	ACT	CTC
	 V	 V		 Q	 E	P	 s	L		v		. P		G	T	V	T	L
	ארר	тст	63 GCT	тсс	AGC	72 ACT	GGG	GCA	81 GTC	ACC	AGG	90 GGT	TAC	TAT	99 CCA	AAC	TGG	108 TTC
	T	 C	 A	 S	 S		 G	Α	v	T	R	G	Y	Υ	P	N	w	F
			<del></del>							ori -		1 4 4			153	<del></del>		162
	CAG	CAG	117 AAG	CCT	GGA	126 CAA	GCA	ccc	135 AGG	GCA	CTG	ATT	TAT	AGT		AAC	AAA	AAA
	- <del></del>	 Q	 К	 P	 G	Q			R				Y	s	T	N	K	ĸ
	_	-										100		<del></del>	207	· CDR	2 —	216
			171	3.00	C C T	180	ccc	ጥጥር	189	GGC	TCC	198 CTC	CTT	GGG	GGC	AAA	GCT	
	CAC	TCC	TGG	ACC														
	Н	s	W	T	P	Α	R	F	S	G	S	L	L	G	G	К	A	Α
		<b>→</b>										252			261			270
	CTG	ACA	225 CTG	TCA	GGT	234 GTG	CAG	ССТ	GAA	GAC	GAG	GCT				TGC		
	 L	 Т	 L	 S	 G	 V			 E							С		L
			279			288			297			306		n n G			← GTC	324 CTA
	TAC	TAT	GGT	GGT	GCT	CAA	CTC	GTA	TTC	GGC	GGA							CTA
	Y	Y	G	G	Α	Q	_	v	F	G	G	G	Т	K	I.	T	V	r
			222		₹3			<del></del>										
	CGT	CAG	333 CCC	3 '														
	R	Q	P															

#### Fig. 10a

#### LD2-11-VH sequence

		9			18			27				~ · ·	225	45	ccc	TCC.	54
CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	GTG	GTC	CAG					
Q	v	ĸ	L	L	E	S	G	G	G	٧	٧	Q	P	G	G	S	L
AGA	CTC	63 TCC	TGT	GAA	72 GCG	TCT	GGA	81 TTC	ACC	стс	90 AGA	AGT	TCT	99 GGC	ATG	CAC	10: TG:
 R		s	c		A	s	G	F	T	L	R		s	G	М	Н	W
GTC	CGC	117 CAG	GCT	сст	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	·		CDR1 153 ATA			16: GA
V	R	Q	Α	P	G	K	G	L	E	W	V		L		W W	F	D
GGA	AGT	171 ATC	AGA	TCG	180 TAT	GCA	GAA	189 TCC	GTG	AAG	198 GGC			207			21
<b>-</b>	s	I	R	s	Y	A	E	s	v	ĸ	G	R	F	T	1	s	R
GAC	ACT	225 TCC	AAG	AAC	- CDF 234 ACC	R2	TAT	243 CTC	CAA	ATG	252 CGC	AGT	CTG	261 AGT	GCC	GAC	27 GA
	 T	 S	 К		 T	L	Y	L	Q	М	R	S	L	s	A	D	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGC	AGG	32 TA
	 А	v	Υ	Y	c	A	R	D	K		v					R	Y
								<del></del>				- CD	R3 —				
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA	GGG	ACC	360 ACG	GTC	ACC	369 GTC	TCC	TCA	3
N	Υ	_		D		W	 G	ĸ	G	Т	T	v	T	v	s	s	
		_ CD	R3 —														

# Fig. 10b

#### LD2-11-VL sequence

			9			18			27			36	ልጥል		45			54
•	GTG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	CGA	GAC	AGA	GTC	ACC
	 V	 L	 T	 Q	 S	P	 S	s	L	s	A	S	I	R	D		V	T
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AAC	81 ATT	GGC	AGT	90 TAT	TTA	AAT 	99 TGG 			
	 I	 T	c	R	A	s	Q	N	I	G	S	Y	L	N	W	Y	Q	Н
	AAA	CCA	117 GGG	ACA	GCC	126 CCT	AAA	CTC	CDRI	ATC	TAT	144 GCT	GTA	TCC	153 GCT	TTG		AGT
	<b>-</b> К	P	G	T	Α	P	K	L	L	I	Y	Α		S	— сп	L B2 —	_	S →
	GGG	GTC	171 CCA	TCG	AGG	TTC	AGT	GGC	AGT	AGA	TCT	198 GGG	ACA	GAT	207 TTC	ACT	CTC	216 ACC
	G	v	-										T				L	Т
	ATC	AGC	225 AGT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
													Y					Y
			270			288			297			306	CAG		315		1	
	 S	 P	P DR3 -	Y		 F	<b>-</b>						Q					

#### Fig. 11a

#### LD2-14-VH sequence

		9			18			27			36			45			5
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CT
Q					E	 s	 G	G	G	v	v	Q	P	G	G	s	L
AGA	GTC	63 GCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AAT	<b>TTT</b>	99 GGC	ATG	CAC	10 TG
 R		 A	c	v	Α	s	G	F	T	r	R				M	Н	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	GCT		CDR1 153 ATT			16 GA
v	R	Q	A	P	G	K	G			W	٧		F			-	C
GCA	AGT	171 AAT	AAA.	GGA	180 TAT	GGA	GAC	189 TCC	GTT.	AAG	198 GGC			207 ACC			21
	<b>S</b>	N	ĸ	 G	Y	G	D	S	v	ĸ	G	R	F	T	٧	S	F
GAC	AAT	225 TCC		AAC	234		TAT	243 CTG	CAA		252 AAC	GGC	CTG	261 AGA	GCC	GAA	27 GA
		 S	 K	 N				L		M	N	G	L	R	Α	E	D
ACG	GCT	279 GTA	TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGT	AGA	32 TA
т		v	 Y	Y	c	Α	R	E	К	Α	V	R	G	I	S	R	Y
		222			342			351			360 ACG	GTC	R3 - ACC	369 GTC	TCC	TCA	3 '
N	Y	Y CDR3	M 	D	∨ 	W	G	K	G	T	T	V	Т	V	S	S	

## Fig. 11b

#### LD2-14-VL sequence

	GTG	ρτα	9 ACC	CAG	тст	18 CCA	TCC	TCC	27 CTG	TCT	GCA	36 TCT	GTG	GGA	45 GAC	AGA	GTC	ACC
,		 M	 T	Q	 s						Α	s	v	G	D	R	V	T
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	ATC	AAC	90 AAT	TTA	AAT	TGG		CAG	108 CAG
		 T	С	R	A	S	Q ?	s	I	I	N	N	L	N	W	Y	Q	Q
				<del></del>				стс	CDR.	ATC	TAT	1 // /		TCC	153 AGT	TTG		162 AGT
			 G					L				Α		S	S	L	Q	S
	GGG	GTC	171 CCT	TCA	AGG	TTC	CGT		AGT	GGA		198 GGG	AGA	GAT	207 TTC	ACT		216 ACC 
	 G	v	P					G								T		_
	GTC	ACC	225 AGT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	
	 V		 S		Q			D								Q	S	Y
	AGT	ACC	279 CTG	TGG	acg	288 TTC	: GGC	CAA	297 . GGG	ACC	AAG	306 GTC	G GAA	ATC	315	•		
	 S	т		w	т	F		Q		T	К	V	Ε	I	K			
			- CD	R3 —		→												

#### Fig. 12a

#### LD2-17-VH sequence

CAG (	GTG	9 AAA	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG
0	 V		 L			 S	G	G		v	٧	Q	P	G	G	s	L
AGA	CTC	63 TCC	TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	TTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	108 TGG
 R			 C							F	R	s	Y	G CDR1	M	H ——→	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	aag	GGC	CTG	GAG	TGG	144 GTG	GCT		153		TTT	162 GAT
	 R		 A								V	Α	F	I	W	F	D
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	TCC	GTG	AAG	198 GGC	CGA		207			216 CGA
	 s		 К	 G	 Y	v	D	s	v	K	G	R	F	T	I	S	R
				AAC				243 CTG	CAA	ATG	252 AAG	AGC	CTG	261 AGA	GCC	GAG	270 GA0
		 S		 N				L			К		L	R		E	D
•				TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306	CGG	GGA	315 ATC	AGT	AGA	32 TA
							R	E	К	A	L	R	G	I			Y
AAC	TAT	333 7AT 7	B C CTG	G GAC	342 GTC	TGG	GGC		GGG		260	CDRS GTC		369	TCC	TCA	. 3
 N	 Y	Y		D	v		. <u></u>					V		v		S	

#### Fig. 12b

#### LD2-17-VL sequence

GTG.	ATG	9 ACC	CAG	TCT	18 CCA	TTC	TCC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	GAC	AGA	GTC	ACC
			Q									v	G		R	v	T
											90 TTT	TTA	AGT	99 TGG	TAT	CAG	108 CAG
				Δ	 S	0	N	I	R	s	F	L	S	W	Y	Q	_
			<del></del>					CI 135 CTG	ATC		144			153 AGG			162 AGT
	 P									Y	Α	Α	S		L	Q	S
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGG		108		GAT	207 TTC	ACT	CTC	216 ACC
G	v	P				S						T	D	F	Т	L	T
ATC	AGC	225 ACT	CTG	CAA	234 CCT	GAA	. GAT	243 TTT	GCG	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
 I											Y			Q	Q	<u>.</u>	Y
AGT	GCC	279 : CCI	TGG	, ACG	288 TTC	: GGC	: CAA	297 . GGG	ACC	: AAG	306 CTG	GAA	ATC	315 AAA			
s	A	 P		Т							L			К			

#### Fig. 13a

#### LD2-20-VH sequence

		۵			18			27			36			45			54
CAG	GTG	AAA	CTG	CTC	GĀG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTC
		 К	 L	 L	E	s	G		G		V	Q	P		G	s	L
AGA	CTC	63 TCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TCC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	10: TG:
 R		5		v	A	s	G	F	T	s	R	s <del>←</del>				H →	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	135 CTG	GAG	TGG	144 GTG			153			16 GA
v		Q		P	G	K	G	L	E	W	V	Α	F	I	W	F	D
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	TCC	GTG	AAG	198 GGC		TTC	207			21
 G	 S			 G	Y	v	D	\$	٧	K		R			I	S	R
GAC	AAT	225 TCC		AAC			TAT	243 CTG	CAA		252 AAG	AGC	CTG	261 AGA	GCC	GAG	27 GA
 D	 N	 S	 К	N		L		L			к			R			ב
ACG	GCT	279 GTA	) X TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGT	AGA	32 TA
					C			E	К	Α	L	R	G	I	S		
AAC	TAT	333 TAC	B CCTC	G GAC	342 GTC	: TG	GGC	263			360	<b>,</b>		369	1	TCA	. :
 N	Y	Y		D	v			K				v		٧			

## Fig. 13b

#### LD2-20-VL sequence

	٥			18			27			36			45			54
ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
 M	 T	Q	s	P	s	<b>5</b>	L	s	Α	s	v	G	D	R	v	T
ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	AGC	AGC	90 TAT	TTA	AAT	99 TGG	TAT	CAG	108 CAG
 T		 R	Α	 S	Q	s	I	s	S	Y	L	N	W	Y	Q	Q
CCA	117 GGG		GCC	126			125			144			153	TTG	CAA	16: AG
 P	 G		 А	P	ĸ	L	L L	1	Y	A	A	S	S	L	Q	S
GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198			207			21
v	P	s	R	F	s	G	S	G	S	G	T	D	F	T	L	T
AGC	225 AGT	СТG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	27 TA
			Q	P					T	Y	Y	С	Q	Q	S	Y
ACC	279 CGA	TTC	ACT	288 TTC	GGC	CCT	297 GGG	ACC	AAA							
т	 R	 F	 Т	 F	 G	 P	 G	 T	 К			 I	к			
	M ACT T CCA P GTC V AGC	M T  63 ACT TGC  T C  117 CCA GGG  P G  171 GTC CCA  V P  225 AGC AGT  S S	ATG ACC CAG  M T Q  63  ACT TGC CGG  T C R  117  CCA GGG AAA  P G K  171  GTC CCA TCA  V P S  AGC AGT CTG  S S L	ATG ACC CAG TCT  M T Q S  ACT TGC CGG GCA  T C R A  117 CCA GGG AAA GCC  P G K A  171 GTC CCA TCA AGG  V P S R  AGC AGT CTG CAA  S S L Q	ATG ACC CAG TCT CCA  M T Q S P  63 72  ACT TGC CGG GCA AGT  T C R A S  117 126  CCA GGG AAA GCC CCT  P G K A P  171 180  GTC CCA TCA AGG TTC  V P S R F  AGC AGT CTG CAA CCT  S S L Q P	ATG ACC CAG TCT CCA TCC  M T Q S P S  ACT TGC CGG GCA AGT CAG  T C R A S Q  CCA GGG AAA GCC CCT AAG  P G K A P K  GTC CCA TCA AGG TTC AGT  V P S R F S  AGC AGT CTG CAA CCT GAA  S S L Q P E  ACC CGA TTC ACT TTC GGC	ATG ACC CAG TCT CCA TCC TCC  M T Q S P S S  ACT TGC CGG GCA AGT CAG AGC  T C R A S Q S  CCA GGG AAA GCC CCT AAG CTC  P G K A P K L  GTC CCA TCA AGG TTC AGT GGC  V P S R F S G  AGC AGT CTG CAA CCT GAA GAT  S S L Q P E D  ACC CGA TTC ACT TTC GGC CCT	ATG ACC CAG TCT CCA TCC TCC CTG  M T Q S P S S L  ACT TGC CGG GCA AGT CAG AGC ATT  T C R A S Q S I  117 CCA GGG AAA GCC CCT AAG CTC CTG  P G K A P K L L  GTC CCA TCA AGG TTC AGT GGC AGT  V P S R F S G S  AGC AGT CTG CAA CCT GAA GAT TTT  S S L Q P E D F  ACC CGA TTC ACT TTC GGC CCT GGG	ATG ACC CAG TCT CCA TCC TCC CTG TCT  M T Q S P S S L S  ACT TGC CGG GCA AGT CAG AGC ATT AGC  T C R A S Q S I S  CDR1  117  CCA GGG AAA GCC CCT AAG CTC CTG ATC  P G K A P K L L I  GTC CCA TCA AGG TTC AGT GGC AGT GGA  V P S R F S G S G  AGC AGT CTG CAA CCT GAA GAT TTT GCA  S S L Q P E D F A  ACC CGA TTC ACT TTC GGC CCT GGG ACC  279  ACC CGA TTC ACT TTC GGC CCT GGG ACC	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA  M T Q S P S S L S A  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC  T C R A S Q S I S S  CDR1  117  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT  P G K A P K L L I Y  GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT  V P S R F S G S G S  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT  S S L Q P E D F A T  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT  M T Q S P S S L S A S  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT  T C R A S Q S I S S Y  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCT  P G K A P K L L I Y A  171 180 189 198  GTC CCA TCA AGG TTC AGT GGC AGT GGG  V P S R F S G S G S G  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC  S S L Q P E D F A T Y  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA  M T Q S P S S L S A S V  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT TTA  T C R A S Q S I S S Y L  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCA GCA  P G K A P K L L I Y A A  171 180 189 198  GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA  V P S R F S G S G S G T  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC  S S L Q P E D F A T Y Y  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT	ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA  M T Q S P S S L S A S V G  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT TTA AAT  T C R A S Q S I S S Y L N  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCT GCA TCC  P G K A P K L L I Y A A S  GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT  V P S R F S G S G S G T D  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT  S S L Q P E D F A T Y Y C  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC  M T Q S P S S L S A S V G D  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT TTA AAT TGG  T C R A S Q S I S S Y L N W  CDR1  117  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCT GCA TCC AGT  P G K A P K L L I Y A A S S  GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC  V P S R F S G S G S G T D F  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA  S S L Q P E D F A T Y Y C Q  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA  M T Q S P S S L S A S V G D R  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT TTA AAT TGG TAT  T C R A S Q S I S S Y L N W Y  CDR1  117  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCT GCA TCC AGT TTG  P G K A P K L L I Y A A S S L  GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT  V P S R F S G S G S G T D F T  AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG  S S L Q P E D F A T Y Y C Q Q  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'	ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC  M T Q S P S S L S A S V G D R V  ACT TGC CGG GCA AGT CAG AGC ATT AGC AGC TAT TTA AAT TGG TAT CAG  T C R A S Q S I S S Y L N W Y Q  CDR1  117  CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCA GCA TCC AGT TTG CAA  P G K A P K L L I Y A A S S L Q  TTO CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC  V P S R F S G S G S G T D F T L  ACC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT  S S L Q P E D F A T Y Y C Q Q S  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'  ACC CGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA 3'

# Fig. 14a

#### LD1-6-17-VH sequence

CAG	GTG	9 AAA	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	ССТ	45 GGG	AGG	TCC	54 CTG
									G			Q	P		R	s	L
AGA	СТТ	63 TCC	TGT	GCA	72 GCG	TCT	GGA	81 TTT	ACC	TTC	90 AGT	AGC	TAT	99 GGC		CAC	108 TGG
										F	S	s	Y	G	М	H →	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG			153		TTT	
v 	 R	Q	A	P	G	K	G	L	E	W	V	Α	D	I	w CDD1	F	D
GGA	GGT	171 AAT	AAA		TAT	GCA	GAC	TTC	GTG	AAG		CGA	TTC	207 ACC	ATC	TCC	216 AGA
G	G	N								· K	G	R	F	T	I	S	R
GAC	AAT	225 TCC			224	GTG		243		ATG	252 AAC	AGC	CTG	261 AGA	GTC	GAG	270 GAC
D	N	 S	ĸ	N	T	v	Y	L	Q	М	N	S	L	R	V	E	D
ACG	GCT	279 <b>GT</b> G	TAT	TAC	288 TGT	GCG	AGG	297 GAT	TAC	TAT	306 AGC	GTT	ACT	315 AAG	AAA 	CTC	324 AGA
т	A	v	Y	Y	С	A	R	D	Y	Y	S	V	Т			L ——	R
CTC	CAC	333 TAC	TAC	TAC	342 TAC	ATG	GAC	351			360			369		ACC	378
L	Н	Y		Y DR3		M	D	v	~ ₩ →	G	к	G	Т	T	v	Ţ	V

TCC TCA 3'

<sup>. . .</sup> 

#### Fig. 14b

#### LD1-6-17-VL sequence

GTG .	ATG	9 ACC	CAG	TCT	18 CCA	TCC	TCC	CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
 V	 М			 S										D	R	V	T
ATC	ACT	63 TGC	CGG	GCA	AGT	CAG	GGC	ATT	AGA	AAT	90 GAT	TTA	ACC	99 TGG	TAT	CAG	108 CAA
	т		 R	Α	s	0	G	I	R	N	D	L	T	W	Y	Q	Q
			<del></del>					CDR 135 CTG	ATC	TAT	144 GCT		,	,	TTA	CAA	162 AGT
 K	 P										Α	Α	S	N	L	Q	S
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGC	GGC	AGT	GGA		198 GGC	ACA	GAT		ACT	CTC	210 ACC
G	v	P	s	R	F	s	G	S	G	S	G	Т	D	F	Т	ŗ	Т
ATC	AGC	225 AGC	CTG	CAG	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAT	TAC	TGT	261 CTA	CAA	GAT	27 AA
		 s												L	Q		N
AAT	TTC	279 : CCG	TAC	: ACT	288 TTT	GGC	CAG	297 GGG	ACC	AAG	306 CTG	GAG	ATC	315 AAA			
	 F	P	Y	T	F				T								

#### Fig. 15a

# LD1/2-6-3-VH sequence

		9			18			27		cmc	36	CNC	cce	4 5 GGG	GGG	TCC	CT.
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC								
 Q	v	 К.	L	L	E	s	G	G	G	v	v	Q	P	G	G	S	L
AGA	GTC	63 GCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AAT	TTT	99 GGC	ATG	CAC	10 TG
 R					 A	s		F			R		F	G	M	Н	W
			-		126 GGC	AĄG	GGG	135 CTG	GAG	TGG	144 GTG	GCT		CDR1 153 ATT		TTT	16 GF
	 R	0	 A			- <b></b>	 G		E		V		F	I	W	F	I
;		_		_	180 TAT	GGA	GAC	TCC	GTT	AAG	198 GGC			207 ACC	GTC	TCC	2: A
 A	 S			<b>-</b>	Y	G						R	F	T	V	S	
		225 TCC	AAG			R2 CTC				ATG	252 AAC	GGC	CTG	261 AGA	GCC	GAA	2 G
		 s					Y			М	N	G	L	R	Α	E	
-		_				GCG	AGA	297 GAG	AAG	GCG	GTT	CGG	GGA	315 ATT	AGT	AGA	3 . T
		v				Α	R	E	К	Α	v	R	G	I	S 	R	
_						: TGG	GGC	351 AAG	GGG		360 ACG	ı	ACC	369 GTC	TCC	TCA	. 3
 N	Υ	 Y		D	<del>-</del>		G				Т			V	S	S	

#### Fig. 15b

## LD1/2-6-3-VL sequence

	GTG	እጥር	9	CNG	ጥርጥ	18 CCA	TCC	TCC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
5'	 V	A1G 	 T	 0	 s	 P			 L			 s	v	 G	D	R	v	T
	•	•	_	-				AGC	0.1	ATC		90 TAT	TTA	AAT	99 TGG	TAT	CAG	108 CAC
		 T		R	Α	s	Q	s	I	I	R	Y	L	N	W	Y	Q	Н
	AAA	CCA	117 GGG	<b>←</b>	GCC				125		CAT	144	GCA	TCC	153 AGT	TTG	CAA	162 AGT
	 K	 Р	 G		 А	P	K	L	L	I	Н	T	Α		s - CD		Q	<del></del> →
	GGG	GTC	171 CCG	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GTA	TCT	198 GGG	ACA		207		CTC	
	- <b>-</b> -	v	P	s	R	F	s	G	S	V	S	G	T	D	F	T,	L	T
	ATC	AGC	225 AGT	CTG	. CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
		 S			 Q								Y				S	Y
	ACT	' ACC	279 : CCG	TAC	: ACT	288 TTI	GGC	: CAG	297 GGG	ACC	: AAG	306	CAG	ATC	315 AAA			
	Ţ	T		Υ Υ	т ——	F	G						Q					

## Fig. 16a

# LD1/2-6-33-VH sequence

5'	CAG	GTG	9 AAA	CTG	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	CTG
,		 V				 Е	 s	G	G	G	v	v	Q	P	G	G	S	L
	-			TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	TTC	90 AGG	AAT	TTT 	99 GGC	ATG		108 TGG
			 A		v	 A	 S	G	F		F		N	F	G CDR1	M	H	W
	GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	CTG	GAG	TGG	144 GTG			153			162 GAT
			 Q		 P		к				W		Α	F		W	F	D
	GCA	AGT	171 AAT	a.a.a	GGA	180 TAT	GGA	GAC	189 TCC	GTT	AAG	198 GGC			207			216 AGA
		 s	 N	 K									R			٧	S	R
						- CDI	R2					<del>&gt;</del>			261	GCC	GAA	270 GAC
		 N	 S	 К	 N	т		Y		Q		N		L	R		E	D
	_	•	279 GTA	TAT	TAT	288 TGT	. ece	AGA	297 GAG	, AAG	GCG	306 GTT	cGG	GGA	315 ATT	AGT	AGA	324 TAC
	т				Y				E	к	Α	٧	. R	G		S		Y
	АА	TAC	333 TAC	S C ATC	G GAC	342 GTC	t TGG	GGC				260	١		369	TCC	TCA	3'
	N	Y	Y	м 3 —	D	v	w		К			Т	V	T	٧		S	

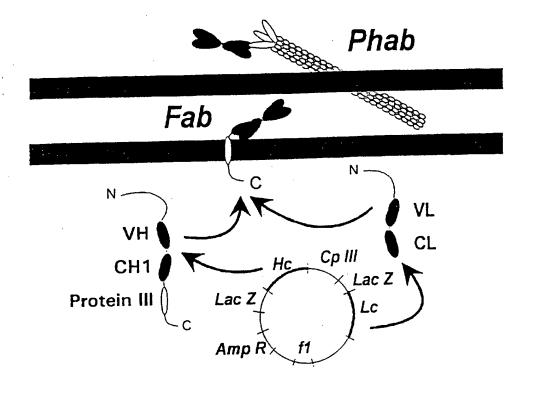
#### Fig. 16b

# LD1/2-6-33-VL sequence

5,	GTG.	ATG	9 ACC	CAG	TCT	18 CCA	TCC	TTC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC		GTC	
,					<b>-</b> S							s	v	G	D	R	v	T
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	ATC	AGA	107	TTA	AAT	TGG	TAT	CAG	108 CAC
	- <b></b>	 Т	_ <del>_</del> _					S	I	I	R	Y	L	N	W	Y	Q	H
				<del></del>					— CI 135 CTG	ATC		1//			153		CAA	162 AGT
					 A							Α	Α	S	S	L	Q	S
								GGC	189 AGT		тст	100			207	ACT	CTC	216 ACC
	 G		- <del>-</del> -	 S	R	F	S	G	S	V	, S	G	T	D	F	T	L	Т
												252			261 CAA	CAG		270 TAC
		 S	 S	 L	Q	P	E	D	F	Α	Т	Ĭ.	Y	С	Q		S	Y
									207	,		30€	5		315 AAA			
	 T	<del>-</del> -	 P	Y	T								Q					

Fig. 17

# The pComb3 Expression System



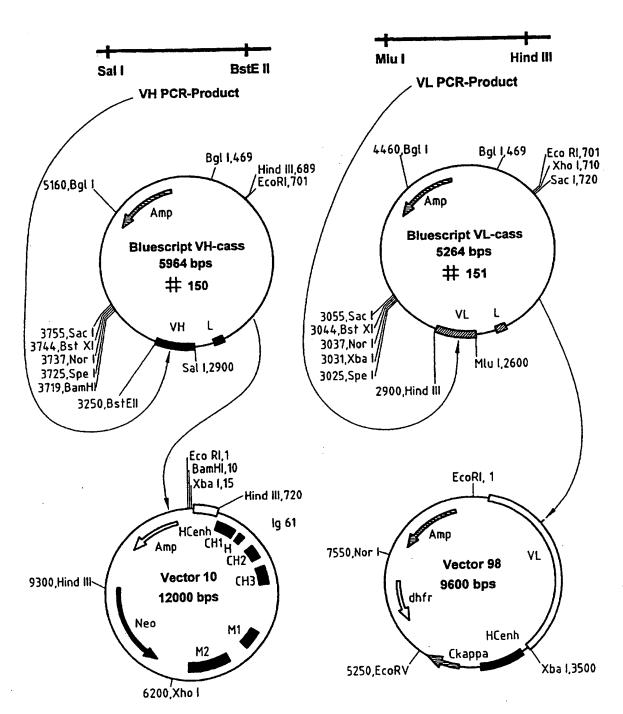


FIG. 18

FIG. 19

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A. CLASSIF IPC 6	FICATION OF SUBJECT MATTER C12N15/13 C12N15/62 C	:07K16/34	A61K39/395	G01N33/80
According to	o International Patent Classification (IPC) or to both natio	nel classification an	d IPC	
	SEARCHED			
Minimum do IPC 6	commentation searched (classification system followed by C12N C07K A61K G01N	y olassification symb	idis)	
Documentati	ion searched other than minimum documentation to the e	extent that such doo	uments are included in the	sfields searched
Electronic de	sta base consulted during the international search (name	e of data base and,	where practical, search te	rme used)
C. DOCUME	ENTB CONSIDERED TO BE RELEVANT			
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* Special cat	egories of cited documents :	e70 lake	والعراطات المساحدة	r the international filing date
conside "E" earlier de filing da "L" documer which is obtation "O" documer other m "P" documer later the	nt which may throw doubts on priority claim(s) or a cited to establish the publication date of another or other special reason (as specified) intreferring to an oral disclosure, use, exhibition or neans at published prior to the international filing date but an the priority date claimed	or cit in "X" doc ce in "Y" doc ce dc m in	priority data and not in our ted to understand the prino vention ourment of particular relevan unnot be considered novel volve an inventive stap who unment of particular relevan unnot be considered to tree ocument is combined with course the art. ourment member of the sam unment member of the sam	nflict with the application but interest theory underlying the noe; the claimed invention or cannot be considered to en the document is taken alone noe; the claimed invention sive an inventive step when the one or more other such doou- ing obvious to a person skilled se patent family
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Name and m	nailing address of the ISA  European Patent Office, P.B. 5818 Patentiaan 2  NL - 2280 HV Rijawijk  Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  Fax: (+31-70) 340-3016	Aut	Müller, F	

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